REFERENCES

Callow, E. H. (1929). *Biochem. J.* **23**, 648.
Campbell, W. R. & Hanna, M. I. (1937). *J. biol. Chem.* **119**, 15.
Cohn, C. & Wolfson, W. Q. (1947). *J. Lab. clin. Med.* **32**, 1203.
Cuthbertson, E. M. & Greenberg, D. M. (1945). *J. biol. Chem.* **160**, 83.
Darrow, D. C. (1945). *New Engl. J. Med.* **233**, 91.
Darrow, D. C. (1946). *J. clin. Invest.* **25**, 324.
Dicker, S. E. (1947). *Proc. R. Soc. Med.* **40**, 353.
Dicker, S. E., Heller, H. & Hewer, T. F. (1946). *Brit. J. exp. Path.* **27**, 158.
Eichelberger, L. (1939). *J. biol. Chem.* **128**, 137.
Eichelberger, L. (1941). *J. biol. Chem.* **140**, 467.
Fenn, W. O. (1936). *Physiol. Rev.* **16**, 450.
Fisher, R. A. (1944). *Statistical Methods for Research Workers.* Edinburgh and London: Oliver and Boyd.
Fisher, R. A. & Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research.* Edinburgh and London: Oliver and Boyd.
Frisch, R. A., Mendel, L. B. & Peters, J. P. (1929). *J. biol. Chem.* **84**, 167.
Fulton, J. F. (1946). *Howell's Textbook of Physiology*, 15th ed., p. 941. Philadelphia and London: Saunders.
Gamble, J. L., Ross, S. G. & Tisdall, F. F. (1923). *J. biol. Chem.* **57**, 663.
Gounelle, H., Bachet, M. & Marche, J. (1942). *C.R. Soc. Biol., Paris*, **136**, 421.
Govaerts, P. & Lequime, J. (1942). *Bull. Acad. Méd. Belg.* **7**, 260.
Harrison, H. E., Darrow, D. C. & Yannet, H. (1936). *J. biol. Chem.* **113**, 515.
Hastings, A. B. & Eichelberger, L. (1937). *J. biol. Chem.* **117**, 73.
Hawk, P. B. & Bergeim, O. (1942). *Practical Physiological Chemistry*, 11th ed., p. 888. London: Churchill.
Hevesy, G. & Rebbe, O. (1940). *Acta physiol. Scand.* **1**, 171.
Höber, R. (1947). *Physical Chemistry of Cells and Tissues.* London: Churchill.
Hoch, H. & Marrack, J. (1945). *Brit. med. J.* ii, 151.
Kerr, W. J., Hurwitz, S. H. & Whipple, G. H. (1918). *Amer. J. Physiol.* **47**, 356.
Keys, A. & Taylor, H. (1935*a*). *J. biol. Chem.* **109**, 47.
Keys, A. & Taylor, H. (1935*b*). *J. biol. Chem.* **109**, 55.
Keys, A., Taylor, H. L., Mickelsen, O. & Henschel, A. (1946). *Science*, **103**, 669.
Kohman, E. (1920). *Amer. J. Physiol.* **51**, 378.
Kramer, B. & Tisdall, F. K. (1921). *J. biol. Chem.* **46**, 339.
Landis, E. M. (1930). *Amer. J. Physiol.* **93**, 353.
McCance, R. A. & Shipp, H. L. (1931). *Biochem. J.* **25**, 449, 1845.
McCance, R. A. & Shipp, H. L. (1933). *Spec. Rep. Ser. med. Res. Coun., Lond.*, **187**, 1.
Manery, J. F. & Bale, W. R. (1941). *Amer. J. Physiol.* **132**, 215.
Manery, J. F. & Haege, L. F. (1941). *Amer. J. Physiol.* **134**, 83.
Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
Marrack, J. & Hewitt, L. F. (1927). *Biochem. J.* **21**, 1129.
Medical Research Council War Memorandum no. 14 (1945). *Nutritive Values of Wartime Foods*, London: H.M.S.O.
Metcoff, J., Favour, C. & Stare, F. J. (1945). *J. clin. Invest.* **24**, 82.
Meyer, P. (1932). *Ergebn. Physiol.* **34**, 18.
Mollison, P. L. (1946). *Brit. med. J.* i, 4.
Pappenheimer, J. R. & Soto-Riviera, A. (1947). XVII *Int. physiol. Congr.* p. 285. Abstracts of Communications.
Peters, J. P. (1944). *Physiol. Rev.* **24**, 491.
Starling, E. H. (1895). *J. Physiol.* **19**, 312.
Truax, F. L. (1939). *Amer. J. Physiol.* **126**, 402.
Verney, E. B. (1926). *J. Physiol.* **61**, 319.
Whitehorn, J. (1921). *J. biol. Chem.* **45**, 449.
Zeldis, L. J., Alling, E. L., McCoord, A. B. & Kulka, J. P. (1945). *J. exp. Med.* **85**, 157.

Changes in the Extracellular- and Intracellular-Fluid Phases of Tissues during Water Diuresis in Normal and Hypoproteinaemic Rats

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

(*Received* 2 *February* 1948)

It has been shown in a previous paper (Dicker, Heller & Hewer, 1946) that the urinary excretion of administered water by protein-deficient rats is delayed, and that the total amount of water excreted in 3 hr. is lower than that of normal animals. There would seem to be at least three likely explanations for the abnormal and delayed diuretic response observed in these rats: (*a*) a slower rate of water absorption from the alimentary canal; (*b*) an increased 'preparedness' of extrarenal tissues to retain water, resulting in a decrease of the plasma water load; (*c*) a failure of the renal tubules to reduce the rate of water reabsorption as a response to the increased water load; or a combination of these factors.

To investigate these possibilities, the rate of water absorption from the gastro-intestinal tract was estimated according to Heller & Smirk (1932), and the partition of absorbed water between the extracellular-fluid phases of tissues was determined.

METHODS

Experimental animals. Adult male albino rats weighing 265–310 g. were used.

Diets. The standard diet (*ST*) and the vegetable low-protein diet (*TT*) conformed to the description given in the previous paper (Dicker, 1948*a*).

Procedure. Normal rats were fed on a standard diet (*ST*) for several weeks. Food, but not water, was withheld 24 hr. before the diuresis experiments. The animals were then given water to the extent of 5% of their body weight by stomach tube, and were killed 15, 30, 45, 60, 75, 90 and 120 min. after the administration.

Another series of rats was fed on diet *TT*. After 6 weeks on this diet, food was withheld for 24 hr. and water to the extent of 5% of their body weight was given. They were killed 15, 30, 45, 60 and 90 min. after the administration.

Immediately before they were due to be killed, the rats were anaesthetized and blood collected from the carotid and jugular was mixed with heparin. Immediately after death the cardia and the distal end of the small intestine (excluding the caecum) were ligatured. The alimentary canal was weighed with its contents. Muscle, liver and brain samples were obtained and the amount of water, Cl^-, Na and K in these tissues estimated.

Chemical methods. Cl^-, Na, K and water estimations in plasma and tissue samples conformed to those described previously (Dicker, 1948*a*). Water content was calculated for fat-free tissues.

Protein concentration in plasma was estimated by two different methods: (*a*) a micro-Kjeldahl method, (*b*) the copper sulphate method for measuring plasma specific gravities (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945). The formula used for the calculation of the plasma protein concentration was that proposed by Hoch & Marrack (1945). The two methods gave results which were not significantly different (Dicker, 1948*b*).

Estimation of the extracellular- and intracellular-fluid phase of tissues. Extracellular and intracellular tissue-fluid phases were calculated from estimation of Cl^- and Na spaces, as in the previous paper (Dicker, 1948*a*), where the identity of these spaces with the true extracellular-fluid phase has also been discussed.

Sampling of rats. The rats were killed in groups consisting of animals with varying absorption times. For statistical treatment of the data, results obtained for animals with identical absorption times were pooled.

Statistical treatment. Results are given as mean and standard errors. Student's '*t*' test for small sample method was used for the significance of means and calculated according to Mainland (1938). *P* values for *t* were obtained from Fisher & Yates's (1943) tables.

RESULTS

Gross post-mortem observation

Rats fed on diet *TT* for 6 weeks had lost about 35% of their body weight; the concentration of proteins in plasma had equally decreased by 35%. No ascites or free fluid was found in any of these animals, even after administration of water, but nearly all those that had received water had abnormally 'wet' tissues. The perirenal and retroperitoneal connective tissues were particularly oedematous, and had a gelatinous appearance. Pressure on these tissues released a substantial quantity of water (up to 1·5 ml.).

In all other respects, these series of protein-deficient animals were comparable with those described in the previous paper (Dicker, 1948*a*).

Water absorption

Fig. 1 shows the rate of water absorption from the alimentary canal in normal and hypoproteinaemic rats. The percentage of body weight of the alimentary canal in normal control rats was 3·6 as compared with 5·7 in controls fed on *TT* diet. In order to render the two curves more easily comparable 2·1 (i.e. the difference between the weights of the alimentary canal in the two series) was deducted from the mean values obtained in the protein-deficient series (Fig. 1).

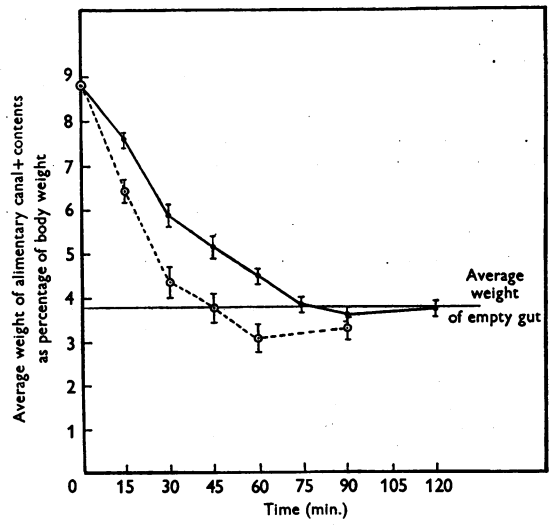

Fig. 1. Average water-absorption curves of rats: (*a*) •—• in normal rats; (*b*) ⊙– – –⊙ in hypoproteinaemic rats. The average weight of the empty gastro-intestinal tract was 3·6% of the body weight in normal control rats as compared with 5·7% of the body weight in controls fed on *TT* diet. In order to render the two curves comparable 2·1% was deducted from the mean values obtained in the protein-deficient series. 5% of the body weight of water was given making 8·6% the starting-point of the absorption curves. The vertical lines represent the standard error.

In normal rats the absorption of 5·0% of body weight of water by the alimentary canal was practically finished in 60–75 min. This is in agreement with Heller & Smirk's (1932) findings. In protein-deficient rats the absorption of the standard amount of water was terminated in 45–60 min. (Fig. 1).

It is thus quite clear that the rate of water absorption from the gut in hypoproteinaemic rats is not slower than that in normal rats, and cannot therefore be the cause of the delayed onset of the water diuresis in these animals.

Changes in water concentration of plasma and tissues in normal and hypoproteinaemic rats

Changes in the water concentration of plasma and tissues after water administration, in both normal and hypoproteinaemic rats, are shown in Table 1. In normal rats the absorption of water resulted in an early increase of the plasma water content ($t = 2{\cdot}666$, $P < 0{\cdot}02$). This increase reached its maximum at about 45 min. and was accompanied by a fall in the plasma protein concentration (Table 1). In hypoproteinaemic rats no significant changes in the plasma water could be found during the 90 min. of observation, nor were there any significant changes in the plasma protein concentration (Table 1). It would seem that the water left the blood plasma at approximately the same rate as it was absorbed from the alimentary canal.

In normal rats the amount of water in muscle and liver was significantly increased 30 and 45 min. after water administration ($t = 3{\cdot}107$, $P < 0{\cdot}001$; and $t = 3{\cdot}779$, $P < 0{\cdot}001$, respectively). The maximum increase in the water load of these tissues occurred at about 60 min.

In protein-deficient rats (*TT*) no significant changes in the amount of water in the liver could be noticed during the whole period of observation. The increase in the amount of water in muscle was delayed: the first significant increase occurred 60 min. after water administration ($t = 2{\cdot}120$, $P < 0{\cdot}05$) as compared with 30 min. in normal rats.

No significant changes in the amount of water in brain were noted in either *ST* or *TT* rats.

Table 1. *The effect of water administration on the water content of plasma muscle, liver and brain of normal and of hypoproteinaemic rats*

(The values are means and standard errors. Numbers of rats in parentheses.)

Stage of observation	Plasma water content (%)	Muscle water content (%)	Liver water content (%)	Brain water content (%)	Plasma proteins (Kjeldahl) (g./100 ml.)
			Normal rats		
Before water administration	91·4±0·13 (27)	75·8±0·32 (27)	71·4±0·31 (27)	83·6±0·24 (11)	7·06±0·092 (27)
15 min. after water administration	91·9±0·11 (20)	76·9±0·30 (20)	71·5±0·44 (20)	83·0±0·26 (9)	6·66±0·120 (18)
30 min. after water administration	92·3±0·10 (18)	77·0±0·15 (18)	72·0±0·33 (18)	83·9±0·34 (7)	6·54±0·182 (18)
45 min. after water administration	93·0±0·18 (18)	77·4±0·16 (18)	72·5±0·32 (18)	83·7±0·27 (7)	6·48±0·120 (18)
60 min. after water administration	92·5±0·26 (16)	77·8±0·21 (16)	72·3±0·32 (16)	84·3±0·76 (6)	6·49±0·108 (16)
75 min. after water administration	92·5±0·17 (16)	77·4±0·11 (16)	71·7±0·27 (16)	84·0±0·48 (6)	6·48±0·058 (16)
90 min. after water administration	92·6±0·13 (12)	76·8±0·13 (12)	72·2±0·30 (12)	83·9±0·31 (6)	6·48±0·075 (12)
120 min. after water administration	92·0±0·09 (12)	76·3±0·23 (12)	71·8±0·30 (12)	83·4±0·15 (6)	6·64±0·190 (12)
			Hypoproteinaemic rats		
Before water administration	93·2±0·16 (29)	77·2±0·28 (30)	74·9±0·39 (30)	83·3±0·41 (12)	5·12±0·137 (29)
15 min. after water administration	93·5±0·14 (10)	77·5±0·21 (10)	74·8±0·54 (10)	83·8±0·67 (10)	4·66±0·101 (10)
30 min. after water administration	93·5±0·16 (12)	77·1±0·82 (12)	74·6±0·60 (12)	83·5±0·26 (8)	4·75±0·130 (10)
45 min. after water administration	93·7±0·16 (16)	77·6±0·50 (16)	74·1±0·48 (16)	83·5±0·33 (6)	4·73±0·163 (16)
60 min. after water administration	93·7±0·14 (20)	78·4±0·28 (20)	75·0±0·34 (20)	83·4±0·92 (8)	4·86±0·136 (20)
75 min. after water administration	—	—	—	—	—
90 min. after water administration	93·3±0·20 (7)	78·2±0·22 (7)	75·4±0·57 (7)	83·4±0·18 (7)	4·93±0·131 (7)
120 min. after water administration	—	—	—	—	—

Changes in the concentration of electrolytes of plasma and tissues in normal and hypoproteinaemic rats

The concentration of chloride in plasma decreased soon after water administration in normal and hypoproteinaemic rats (Tables 2 and 3). The decrease in the plasma chloride concentration lasted for 30 min. in normal rats, and more than 60 min. in *TT* rats; in other words, it covered in both series the period of time elapsing between that of administration of water and that of the onset of water diuresis. The decrease of chloride concentration in the plasma was not the result of a dilution of the plasma. This is seen from the results obtained on rats fed on *TT* diet (Table 1) where the administration of water by stomach tube did not produce an increase of the plasma water content. Nor can the decrease be explained by an escape of chloride into the muscles or the liver (Tables 2 and 3): in both normal and protein-deficient rats the fall in the plasma chloride concentration was concurrent with a fall in the concentration of chloride in the muscle, while the chloride concentration in the liver remained unaffected. The fall in muscle chloride in the two series of rats lasted as long as that in the plasma. Finally, it will be noted (Tables 2 and 3) that variations in plasma chloride were independent of variations in the concentrations of sodium and potassium in the plasma. These findings agree with those of Priestley (1916), Smirk (1932) and Eggleton (1937) which suggest that water diuresis is preceded by passage of chloride into the gut.

Table 2. *The effect of water administration on the concentration of electrolytes of plasma and tissues in normal rats*

(The values are means and standard errors. Number of rats in parentheses.)

Stage of observation	Cl^- (m-equiv./l. water)	Na (m-equiv./l. water)	K (m-equiv./l. water)	Cl^- (m-equiv./l. water)	Na (m-equiv./l. water)	K (m-equiv./l. water)
		Plasma			Muscle	
Before water administration	83·2±2·34 (27)	128·3±7·05 (18)	5·6±0·29 (17)	20·2±0·47 (27)	29·0±1·48 (17)	156·2±7·73 (17)
15 min. after water administration	72·3±1·63 (20)	112·8±4·04 (10)	4·5±0·31 (9)	17·5±0·67 (20)	25·0±2·27 (9)	155·0±4·57 (9)
30 min. after water administration	69·3±2·04 (18)	112·3±4·56 (12)	5·2±0·41 (7)	18·9±0·99 (18)	26·0±2·73 (11)	145·3±2·87 (7)
45 min. after water administration	82·2±2·80 (18)	134·2±5·32 (15)	6·8±0·66 (13)	22·5±0·92 (18)	36·1±1·69 (13)	134·2±5·62 (13)
60 min. after water administration	82·3±5·54 (16)	137·5±3·90 (12)	5·7±0·44 (12)	22·4±1·23 (16)	36·1±1·94 (12)	147·2±4·41 (12)
75 min. after water administration	86·5±2·16 (16)	137·6±5·57 (16)	5·2±0·26 (16)	23·2±0·66 (16)	37·5±1·98 (16)	158·7±4·60 (16)
90 min. after water administration	78·6±2·62 (12)	128·3±3·09 (12)	6·1±0·40 (12)	20·4±0·74 (12)	32·5±1·12 (12)	154·0±8·01 (12)
120 min. after water administration	84·0±1·87 (12)	131·3±4·00 (12)	5·0±0·26 (12)	20·7±0·75 (12)	29·1±1·55 (12)	157·5±3·67 (12)
		Liver			Brain	
Before water administration	33·6±2·80 (11)	31·0±2·09 (10)	97·8±7·63 (10)	42·7±2·89 (9)	49·0±3·56 (10)	124·0±10·50 (10)
15 min. after water administration	29·0±2·74 (9)	38·1±3·71 (9)	98·0±4·56 (9)	35·3±3·56 (8)	46·1±4·13 (8)	137·8±8·17 (9)
30 min. after water administration	34·0±3·83 (7)	45·5±4·12 (7)	94·9±4·90 (7)	33·4±3·37 (7)	46·1±6·20 (7)	132·7±12·20 (7)
45 min. after water administration	47·0±1·83 (7)	55·6±1·72 (7)	106·0±9·36 (7)	39·0±2·29 (6)	53·0±3·35 (7)	124·0±7·56 (7)
60 min. after water administration	42·0±3·80 (6)	51·4±3·39 (5)	115·5±12·10 (5)	37·7±3·26 (6)	55·3±2·80 (6)	127·5±7·78 (6)
75 min. after water administration	38·4±2·33 (6)	55·4±3·09 (6)	126·1±10·30 (6)	37·3±2·20 (6)	55·5±2·14 (6)	131·6±4·74 (6)
90 min. after water administration	40·5±2·76 (6)	49·2±3·05 (6)	116·6±12·46 (6)	38·0±2·81 (6)	51·4±3·81 (6)	146·5±5·18 (6)
120 min. after water administration	34·8±1·92 (6)	35·0±2·14 (6)	127·0±9·74 (6)	40·3±3·55 (5)	53·7±1·60 (6)	130·9±9·17 (6)

Table 3. *The effect of water administration on the concentration of electrolytes of plasma and tissues in hypoproteinaemic rats*

(The values are means and standard errors. Number of rats in parentheses.)

Stage of observation	Cl^- (m-equiv./l. water)	Na (m-equiv./l. water)	K (m-equiv./l. water)	Cl^- (m-equiv./l. water)	Na (m-equiv./l. water)	K (m-equiv./l. water)
		Plasma			Muscle	
Before water administration	85·9±2·77 (29)	108·3±2·12 (11)	7·1±0·58 (11)	46·7±2·44 (29)	69·5±4·22 (11)	96·6±3·84 (11)
15 min. after water administration	75·6±2·45 (10)	100·0±1·62 (10)	6·8±0·38 (10)	41·7±1·87 (10)	59·5±1·51 (10)	105·5±3·89 (10)
30 min. after water administration	63·4±1·03 (12)	97·1±2·20 (8)	7·1±0·82 (8)	37·6±2·68 (12)	61·5±5·92 (8)	93·7±5·99 (8)
45 min. after water administration	71·9±3·08 (16)	104·2±3·40 (6)	5·8±0·21 (6)	42·1±1·76 (16)	61·4±6·30 (6)	109·0±8·27 (6)
60 min. after water administration	79·2±3·20 (20)	110·8±3·55 (8)	6·4±0·53 (8)	51·7±1·82 (20)	76·8±5·00 (8)	97·4±3·20 (8)
90 min. after water administration	86·8±2·90 (7)	113·6±3·42 (7)	6·4±0·12 (7)	47·9±2·66 (7)	64·7±2·23 (7)	109·5±10·81 (7)
		Liver			Brain	
Before water administration	36·4±2·55 (11)	48·7±3·24 (11)	93·8±3·61 (11)	39·1±3·27 (11)	59·5±3·25 (11)	114·4±4·44 (11)
15 min. after water administration	39·5±1·11 (10)	52·3±2·64 (10)	91·8±6·22 (10)	39·5±1·87 (10)	60·9±2·02 (10)	118·5±2·90 (10)
30 min. after water administration	34·6±3·04 (6)	49·1±3·40 (6)	89·7±5·35 (6)	35·7±2·77 (8)	59·1±4·83 (8)	118·8±10·56 (8)
45 min. after water administration	38·6±4·96 (6)	46·6±2·70 (6)	92·5±2·48 (6)	35·1±1·34 (6)	58·6±1·96 (6)	110·5±6·82 (6)
60 min. after water administration	39·3±3·61 (8)	58·5±5·77 (8)	98·5±7·76 (8)	39·5±2·99 (8)	60·9±5·38 (8)	112·6±6·23 (8)
90 min. after water administration	39·0±0·97 (7)	56·1±1·43 (7)	102·2±13·11 (7)	40·0±1·67 (7)	62·8±3·24 (7)	116·0±5·60 (7)

The plasma chloride concentrations were normal again in normal rats 45 min. after water administration, and in hypoproteinaemic rats 60 min. after water administration, and then remained at this level for the remaining period of observation. Chloride concentration in muscle increased over the mean values of control rats, 45 min. after water administration in normal rats ($t=3·010$, $P<0·01$) and 60 min. after water administration in hypoproteinaemic rats ($t=4·031$, $P<0·001$). The increase in the chloride concentration of muscle in the two series corresponded to the onset of water diuresis.

It is thus clear that in normal as well as in hypoproteinaemic rats a water diuresis can be divided into two stages: (*a*) a prediuretic or absorptive period, during which there was a fall of the chloride concentration in both plasma and muscle, independent of changes in their hydration; (*b*) an excretory period with increased water load in plasma and tissues during which the chloride concentration returned, nevertheless, to normal values in plasma and reached higher than normal values in the muscle.

Changes in the extracellular-fluid phase of tissues

The changes in the water content of plasma and the tissues, associated with changes in the concentration of electrolytes, led to variations in the amount of extracellular fluid of tissues.

It would seem from Tables 4 and 5 that the partition of water between the extracellular- and intracellular-fluid phase during a water diuresis differs in normal and hypoproteinaemic animals.

In normal rats three stages could be recognized (Fig. 2): an initial decrease of the extracellular-fluid phase of muscle (up to 30 min. after water administration) followed by a significant increase (45–75 min.) ending in a slow return to normal values (90–120 min.). When the changes of the extracellular-fluid phase are correlated with the rate of renal water excretion, it can be seen that the initial decrease of extracellular-fluid phase corresponded to the prediuretic period and the increase to the peak of the diuresis.

In the hypoproteinaemic rats, although the average changes in extracellular-fluid phase of

Table 4. *The effect of water administration on the extracellular- and intracellular-fluid phases (chloride and sodium) of tissues in normal rats*

(The values are means and standard errors. Number of rats in parentheses.)

Stage of observation	Extracellular-fluid phase (ml./100 g.)		Intracellular-fluid phase (ml./100 g.)	
	Cl^-	Na	Cl^-	Na
Muscle				
Before water administration	16·8±0·50 (27)	16·9±0·53 (17)	59·0±0·50 (27)	58·8±1·11 (17)
15 min. after water administration	15·3±0·27 (20)	15·4±0·47 (9)	61·5±0·40 (20)	61·5±1·23 (9)
30 min. after water administration	18·9±0·90 (18)	18·0±1·00 (11)	58·2±0·85 (18)	59·0±1·04 (11)
45 min. after water administration	20·8±0·85 (18)	21·1±0·86 (13)	56·5±0·89 (18)	56·3±0·88 (13)
60 min. after water administration	20·5±0·88 (16)	20·5±1·18 (12)	57·3±0·93 (16)	57·3±1·13 (12)
75 min. after water administration	20·6±0·66 (16)	20·7±0·70 (16)	56·7±0·63 (16)	56·7±0·64 (16)
90 min. after water administration	18·3±0·80 (12)	20·4±0·78 (12)	58·5±0·78 (12)	56·5±0·82 (12)
120 min. after water administration	17·1±0·49 (12)	16·5±0·74 (12)	59·3±0·59 (12)	59·7±0·80 (12)
Liver				
Before water administration	22·6±2·50 (11)	21·3±1·55 (10)	48·8±2·05 (11)	50·1±2·03 (10)
15 min. after water administration	25·9±2·76 (9)	23·8±2·42 (9)	45·6±1·56 (9)	47·6±1·20 (9)
30 min. after water administration	34·4±3·60 (7)	32·6±3·57 (7)	37·6±3·65 (7)	40·6±3·63 (7)
45 min. after water administration	32·0±1·40 (7)	33·4±3·90 (7)	40·8±1·43 (7)	39·2±3·80 (7)
60 min. after water administration	27·6±2·91 (6)	26·3±4·32 (5)	44·7±3·40 (6)	46·0±4·93 (5)
75 min. after water administration	30·1±2·39 (6)	30·2±3·15 (6)	41·6±1·76 (6)	41·3±3·03 (6)
90 min. after water administration	30·6±1·87 (6)	32·9±2·50 (6)	41·6±3·40 (6)	39·4±2·53 (6)
120 min. after water administration	31·5±1·70 (6)	28·1±1·95 (6)	40·3±1·75 (6)	43·7±2·30 (6)

Table 5. *The effect of water administration on the extracellular- and intracellular-fluid phases (chloride and sodium) of tissues in hypoproteinaemic rats*

(The values are means and standard errors. Number of rats in parentheses.)

Stage of observation	Extracellular-fluid phase (ml./100 g.)		Intracellular-fluid phase (ml./100 g.)	
	Cl^-	Na	Cl^-	Na
Muscle				
Before water administration	44·9±1·90 (29)	43·5±2·08 (11)	32·3±1·64 (29)	33·8±0·70 (11)
15 min. after water administration	41·9±2·16 (10)	38·8±1·24 (10)	35·5±1·86 (10)	38·7±1·24 (10)
30 min. after water administration	44·2±3·41 (12)	42·0±3·90 (8)	32·7±3·00 (12)	35·0±3·72 (8)
45 min. after water administration	46·2±3·51 (16)	44·0±4·66 (6)	31·4±3·40 (16)	33·5±4·12 (6)
60 min. after water administration	49·1±2·42 (20)	51·5±4·67 (8)	30·0±2·40 (20)	26·9±3·67 (8)
75 min. after water administration	—	—	—	—
90 min. after water administration	41·1±2·45 (7)	42·2±2·29 (7)	37·0±2·72 (7)	36·0±2·66 (7)
120 min. after water administration	—	—	—	—
Liver				
Before water administration	35·4±2·66 (11)	32·6±1·91 (11)	39·5±2·52 (11)	42·2±2·14 (11)
15 min. after water administration	39·8±1·22 (10)	37·9±2·75 (10)	35·0±1·15 (10)	37·0±3·03 (10)
30 min. after water administration	40·8±3·14 (6)	33·4±2·38 (6)	33·8±3·12 (6)	41·2±2·62 (6)
45 min. after water administration	39·4±3·42 (6)	32·3±2·02 (6)	34·9±3·59 (6)	41·8±1·87 (6)
60 min. after water administration	40·4±3·66 (8)	37·2±3·76 (8)	34·8±3·54 (8)	38·0±3·67 (8)
75 min. after water administration	—	—	—	—
90 min. after water administration	33·6±1·07 (7)	36·0±1·64 (7)	41·8±0·83 (7)	39·4±1·37 (7)
120 min. after water administration	—	—	—	—

muscle resembled closely, both in magnitude and direction, those observed in the normal animals, such changes cannot be regarded as significant owing to the large standard error of each group (Table 5).

Changes in the intracellular-water phase of tissues

It has been shown in the present series of experiments on normal rats that during a water diuresis the total water load of muscle and its extracellular-fluid phase increased in a parallel manner. But it is not clear whether these simultaneous increases are of the same magnitude, i.e. whether the increase of the extracellular-fluid phase can be accounted for by the increase of the extra water load of the muscle.

Comparing the mean values for extracellular fluid and the total muscle water load (Tables 1 and 4) it will be seen that, whereas the extracellular-fluid phase increased from 16·8 to 20·5 ml./100 g. fat-free muscle (i.e. by 3·7 ml.) in 60 min., that of total water content increased from 75·8 to 77·8 ml./100 g. fat-free muscle (i.e. by 2·0 ml.) in the same time. The differences in the increments represents the changes in the amount of intracellular-fluid phase (Table 4).

It is thus evident that during the second or excretory stage of water diuresis an increase in the extracellular-fluid phase was associated with a decrease in the amount of intracellular fluid with a concurrent increase in the extra water load of the muscle (Fig. 2). In agreement with this finding is the observation that the loss of intracellular water was accompanied by a loss of potassium (Table 2). This finding agrees with that of Gamble, Blackfan & Hamilton (1925), who showed that water diuresis may cause a marked urinary excretion of endogenous potassium.

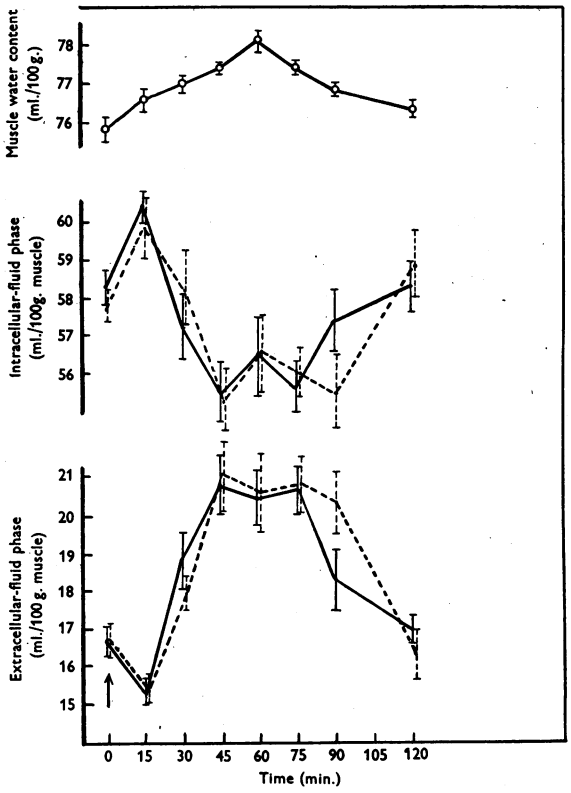

Fig. 2. Changes of total water content and of the extracellular- and intracellular-fluid phases in the muscle of normal rats. At ↑ 5% of body weight of water was administered. •—•, extracellular- and intracellular-fluid phases estimated in terms of chloride space; •- - - -•, extracellular- and intracellular-fluid phases estimated in terms of sodium space; ○—○, total muscle water content. The vertical lines represent the standard error.

In hypoproteinaemic animals, on the other hand, no clear changes in the intracellular-fluid phase of the tissues could be shown. The partition of water between the extracellular- and the intracellular-fluid phases in the tissues during a water diuresis was thus completely different in normal and hypoproteinaemic rats.

DISCUSSION

It would appear that in normal rats well-defined changes in the partition of water between the extracellular- and the intracellular-fluid phase of skeletal muscles corresponded to certain stages in the process of water diuresis. Following the administration of water by stomach tube, and preceding the onset of diuresis, there was a significant decrease in the extracellular-fluid phase of the muscle. When the absorptive period was reaching completion and when the renal excretion of the administered water had started, the well-known increase of the muscle water load was observed. A significant increase in the extracellular-fluid phase concurrent with a decrease of the intracellular-fluid phase could be demonstrated at this stage. When the excretion of the extra water was nearly completed there was a return of the muscle water load and of its extracellular- and intracellular-fluid phases to normal values.

These results compare with Eichelberger's (1941) findings on unanaesthetized dogs, but are in apparent contrast to those of Eggleton (1937) on anaesthetized cats. Eichelberger (1941) injected 0·9% NaCl solution intravenously into dogs, and observed that during the period of full diuresis there was a significant increase in the extracellular-fluid phase of the muscle, and a slight decrease in its intracellular-fluid phase. In other words, the partition of water in Eichelberger's (1941) dogs corresponded to that observed during the excretory stage in the present series of normal rats. Eggleton (1937), on the other hand, found that the injection of 6% of the body weight of water 'into a loop of small intestine brought to the surface through a small abdominal opening' produced an increase in the total muscle water content accompanied by a decrease of its extracellular-fluid phase. It should be pointed out, however, that none of Eggleton's (1937) cats was excreting the administered water; in fact, some of them had their ureters tied.

The difference between Eggleton's (1937) findings on the one hand, and those of Eichelberger (1941) and of the present series of investigations on the other, thus lies clearly in the fact that in Eggleton's (1937) cats water excretion was prevented. It is therefore likely that Eggleton's (1937) findings apply to the prediuretic or reabsorptive period only, a stage characterized by a dilution of the plasma solids and by a fall of the chloride concentration in both plasma and muscles, resulting in a decrease of the extracellular space.

In hypoproteinaemic rats the administration of the standard amount of water did not produce a significant increase of the water level in the plasma, in spite of the fact that water had been absorbed at the same rate at least as by normal rats. During the absorptive period there was a marked fall of the

plasma and muscle chloride concentrations, but in contrast to the normal animals there was no significant decrease in the extracellular-fluid phase of the skeletal muscle. Nor was there any significant increase of the extracellular-fluid phase of muscle during the post-absorptive or excretory period. It would thus seem that the tissue oedema that existed initially in the hypoproteinaemic animals could not be increased to any significant extent by further administration of water.

It has indeed been suggested that in muscles in which the concentrations of chloride and sodium are increased and that of potassium decreased (i.e. in which an increase of the extracellular-fluid phase at the expense of the intracellular-fluid phase obtains) the extracellular-fluid phase should not be identified with an ultrafiltrate of plasma (Manery, Danielson & Hastings, 1938) but with connective tissue diluted with serum filtrate. The extracellular-fluid phase of connective tissue has been estimated to amount to almost 100% (Manery & Hastings, 1939). This interpretation may explain why an increased water load did not produce any significant increase in the extracellular-fluid phase of the muscle.

This interpretation would also provide an explanation for the delayed and diminished water diuresis displayed by hypoproteinaemic rats.

In normal rats the administration of a standard amount of water resulted in a dilution of the plasma, which according to accepted theories is indirectly responsible for the reduction of the rate of tubular water reabsorption. There is a time lag between the height of gastro-intestinal water absorption and the height of the diuresis, i.e. a period during which the water absorbed is lodged in the extracellular-fluid phase of tissues, from where it ultimately returns to the blood stream to be excreted by the kidneys. In hypoproteinaemic rats the administration of a standard amount of water did not result in a further dilution of the plasma: the rate of tubular water reabsorption was thus not affected. This would explain why the water diuresis was delayed in its onset, and why the diuresis was partly regulated by an enhanced glomerular filtration rate (Dicker *et al.* 1946). As the rate at which water was absorbed from the gut was not slower than in normal rats, and as the extracellular-fluid phase of skeletal muscle or liver seemed to be unable to store the amount of water absorbed, it must have been stored elsewhere. Few of the rats in this series showed any free fluid at a post-mortem examination, but nearly all of them displayed perirenal and retroperitoneal connective tissues so much distended with fluid as to give them a gelatinous consistency. In several cases mediastinal connective tissue had the same appearance. It must, therefore, be assumed that in hypoproteinaemic rats water administered by stomach tube is not held in the extracellular-fluid phase of muscle or liver, but that it collects in the deep connective tissue.

SUMMARY

1. A standard amount of water (5% of their body weight) was administered by stomach tube to normal and hypoproteinaemic rats, and the partition of the absorbed water between the plasma and the extracellular- and intracellular-fluid phases of certain tissues (muscle, liver and brain) was investigated.

2. In normal rats the administration of water produced effects which led to the division of the process of water diuresis into three distinct periods:

(*a*) The absorptive period, preceding the onset of diuresis and characterized by a dilution of the plasma accompanied by a fall in the chloride and the sodium concentration of both plasma and tissues, resulting in a decrease of the extracellular-fluid phase.

(*b*) The excretory period, corresponding to the height of the water diuresis accompanied by a marked increase in the chloride and sodium concentration of muscle and a decrease of potassium, resulting in an increase of the extracellular-fluid phase and a decrease in the intracellular-fluid phase of the tissue, i.e. oedema of the tissue.

(*c*) The terminal period: return to normal values for chloride, sodium and potassium concentration in plasma and tissues.

3. In hypoproteinaemic rats water administered was absorbed at least at the same rate as in normal animals. The water absorption was finished in less than 60 min.

4. In hypoproteinaemic rats the administration of water produced a diuresis which was delayed in its onset and diminished in its volume. This abnormal response to water administration could be explained by the following findings:

(*a*) The administration of water did not produce any further increase in the plasma water content.

(*b*) No significant changes in the amount of extracellular-fluid phase per 100 g. fat-free muscle could be found.

(*c*) The water absorbed was 'visibly' collected in the perirenal and retroperitoneal connective tissue, which was so much distended as to have a gelatinous consistency.

The expenses of this investigation were partly defrayed by a grant from the Colston Research Committee, whose help is gratefully acknowledged. The author wishes to thank Miss P. A. Ashby for her technical assistance.

REFERENCES

Dicker, S. E. (1948*a*). *Biochem. J.* **43**, 444.
Dicker, S. E. (1948*b*). *J. Physiol.* **107**, 11*P*.
Dicker, S. E., Heller, H. & Hewer, T. F. (1946). *Brit. J. exp. Path.* **27**, 158.
Eggleton, M. G. (1937). *J. Physiol.* **90**, 465.
Eichelberger, L. (1941). *J. biol. Chem.* **138**, 583.
Fisher, R. A. & Yates, F. (1943). *Statistical Tables for Biological, Agricultural and Medical Research*, 2nd ed. London: Oliver and Boyd.
Gamble, J. L., Blackfan, K. D. & Hamilton, B. (1925). *J. clin. Invest.* **63**, 309.
Heller, H. & Smirk, F. H. (1932). *J. Physiol.* **76**, 1.
Hoch, H. & Marrack, J. (1945). *Brit. med. J.* ii, 151.
Mainland, D. (1938). *The Treatment of Clinical and Laboratory Data.* London: Oliver and Boyd.
Manery, J. F., Danielson, I. S. & Hastings, A. B. (1938). *J. biol. Chem.* **124**, 359.
Manery, J. F. & Hastings, A. B. (1939). *J. biol. Chem.* **127**, 657.
Phillips, R. A., Van Slyke, D. D., Dole, V. P., Emerson, K., Hamilton, P. B. & Archibald, R. M. (1945). *Copper Sulphate Method for Measuring Specific Gravities of Whole Blood and Plasma.* New York: Josiah Macy, jun. Foundation.
Priestley, J. G. (1916). *J. Physiol.* **50**, 304.
Smirk, F. H. (1932). *J. Physiol.* **75**, 81.

Chemical Constitution and Insecticidal Action

1. ORGANIC SULPHUR COMPOUNDS

By W. H. DAVIES and W. A. SEXTON
Research Laboratories, Imperial Chemical Industries Ltd., Blackley, Manchester

(*Received* 5 *March* 1948)

Until the discovery of the newer synthetic insecticides of the chlorinated hydrocarbon class, such as DDT and γ-benzene hexachloride, organic compounds of sulphur had attracted considerable attention, and this work led to the commercial introduction of the long-chain alkyl thiocyanates, to phenothiazine (originally as an insecticide but later developed as an anthelmintic) and more recently to tetraethylthiuram sulphide, an acaricide used in the treatment of scabies and mange. During the last ten years we have made an extensive investigation of the insecticidal action of organic sulphur compounds, in collaboration with the Hawthorndale Laboratory of Jealott's Hill Research Station (Imperial Chemical Industries Ltd.) where the entomological testing was carried out. Although these investigations did not result in the introduction of any new commercial insecticides, nevertheless, marked insecticidal activity was found in certain types, and the correlation of structure with activity presented points of considerable interest. It is the purpose of this communication to record some of our observations on this subject.

METHODS

All compounds were submitted to routine sorting tests, and from the results the more promising were selected for extended evaluation. The extended tests necessitated the preparation of a variety of types of emulsions and dispersions (Collie, Ellingworth & Robertson, 1939; Collie, Davies & Sexton, 1939; Harland & Sexton, 1943) suitable for the practical application of the poisons. This aspect of the research is mentioned only so far as it provides data confirming the higher activities found by the sorting tests and illustrates the selective poisoning effect on insect species.

Contact insecticides. The sorting tests were carried out in the Tattersfield spraying apparatus using aphids (*Macrosiphum* spp. except where otherwise stated, the choice of species being governed by its availability at the time of the test), or adult blowflies (*Calliphora erythrocephala* Meig.) as the test insects. The compound to be tested was dissolved in ethanol, and poured into a 0·1% aqueous solution of a surface-active agent of the sulphonated hydrocarbon type, the concentrations being such that the final spray contained the substance dispersed in 50% ethanol. In certain instances it was necessary to use acetone instead of ethanol. As it was appreciated that the organic solvent might play a significant part in the apparent toxicities, controls were carried out with sprays of similar composition from which the poison had been omitted; the 'absolute mortality' due to the substance under test was calculated by the formula:

$$\text{Percentage absolute mortality} = \frac{(\text{number killed by poison spray}) - (\text{number killed by control spray})}{(\text{total number of insects}) - (\text{number killed by control spray})} \times 100.$$

In spite of this adjustment, the ethanol may have played a part in altering penetration of the insect cuticle by the poison (Hurst, 1943) so that the figures of 'absolute mortality' obtained may have been, considered as absolute values, false. It is felt, however, that for closely related