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Changes in Water and Ion Metabolism and in Kidney Functions During the Development of Oedema in Rats Fed on Protein-deficient Diets

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It was assumed until recently that 'famine oedema' is due to a decrease in the colloid osmotic pressure of plasma as the result of an inadequate intake of dietary protein. Some doubts as to the validity of this conception were raised when cases of 'famine oedema' in human beings were described, in which protein concentration and colloid osmotic pressure of the plasma were found to be normal (Govaerts & Lequime, 1942; Keys, Taylor, Mickelsen & Henschel, 1946). Furthermore, in rats fed on a protein-deficient diet it could be shown that tissue oedema started at a time when the plasma protein concentration, and hence the plasma colloid osmotic pressure, were still normal (Dicker, 1948b); and secondly, that there was no clear correlation between the degree of hypoproteinaemia, the fall of the colloid osmotic pressure of plasma and the magnitude of the extracellular fluid phase of tissues when tissue oedema had fully developed (Dicker, 1948b). Finally, it was found that starved rats developed tissue oedema only when free access to water was allowed, and that the increase of the extracellular space was proportional to the amount of water drunk by the animal (Dicker, 1949b). It is not clear whether the retention of water demonstrated in the latter findings is of renal or extra-renal origin, or whether both factors apply. The present paper attempts to investigate the mechanism of the incipient oedema by correlating changes in water and ion excretion with changes in the extracellular fluid phase of tissues, and by analysing kidney functions by clearance methods.

METHODS

Experimental animals and standard diet (HH). Adult male albino rats weighing 270–350 g. were used. Control rats were given the following diet: casein 25.0, wheat

starch 40.0, hardened ground-nut oil 12.5, dried yeast 15.5, salt mixture (Hawk & Bergeim, 1942) 4.0, cod-liver oil 3.0%. This diet yielded approx. 30 kg.cal./10 g.

Protein-deficient diets. Four different types of diet were used; one yielded the same amount of calories as the standard diet, i.e. approx. 30.0 kg.cal./10 g. (diet *BB*), but contained only 0.5% casein, while the others were low both in protein content and in calorific values. Of the latter, two (*TT* and *FF*) had a water content of about 75%, while the other diet (*DD*) did not contain more water than diet *BB*, i.e. 15.0%.

(a) *Low-casein, high-carbohydrate diet (BB).* Casein 0.5, wheat starch 80.0, hardened ground-nut oil 12.5, salt mixture 4.0 and cod liver oil 3.0%. In addition, each animal received a mixture containing 30 µg. aneurin, 50 µg. riboflavin, 1 mg. nicotinic acid, and 15 µg. of tocopheryl acetate; 10.0 g. of this food yielded approx. 30.0 kg.cal.

(b) *Vegetable low-protein diet (TT).* This diet was the same as that used by Dicker, Heller & Hewer (1946). It had the following composition: fresh turnips 81.5, wheat starch 7.0, hardened ground-nut oil 3.5, salt mixture 4.0 and cod liver oil 4.0%. Vitamins B and E were added in the same amount as in diet *BB*. This diet yielded approx. 8.0 kg.cal./10 g.

(c) *Wet filter-paper diet (FF).* This diet was devised to avoid the use of turnips which introduces a factor difficult to control. It was made up as follows: filter paper 4.7, casein 0.8, wheat starch 7.0, hardened ground-nut oil 3.5, salt mixture 4.0, cod liver oil 4.0%, and was made up to a semi-liquid paste by addition of 76.0% water. Vitamins B and E were added in the same amount as in diet *BB*. This diet is very similar to the vegetable low-protein diet (*TT*), especially with reference to its protein and water content, and had approximately the same calorific value as the diet *TT*.

(d) *Dry filter-paper, low-protein diet (DD).* Casein 1.5, wheat starch 13.3, hardened ground-nut oil 8.0, salt mixture 5.3, cod liver oil 5.3, and finely ground filter paper 66.6%. Additions of vitamins B and E were provided in the same amounts as in diet *BB*.

Analytical procedure. Methods for estimating plasma and tissue-water content, Cl^- , Na^+ and K^+ in urine, plasma or muscle, as well as for the calculation of the extracellular fluid phase of fat-free muscle (as expressed in terms of Cl^- , and Na^+ spaces) were the same as those used in previous papers (Dicker, 1948*b*, 1949*b*). Inulin in plasma and urine was estimated by the method of Smith, Goldring & Chasis (1938), and the diodone iodine content of plasma and urine was determined by Alpert's (1941) method.

Preparation of urine concentrates from normal rats and from rats suffering from hypoproteinaemia. Six to ten rats were kept for 24 hr. in a large metabolism cage, without food, but with free access to water. The urine was collected under paraffin, acidified with 3.0% (w/v) acetic acid, concentrated *in vacuo* at 37.0°, and dialysed for 6 hr. using a sac of cellophan (thickness of cellophan = 0.00072 in.). The course of procedure followed closely that described by Ralli, Robson, Clarke & Hoagland (1945).

Kidney function tests. Renal function was first investigated in rats while on the standard diet (*HH*), and later while on the protein-deficient diets *BB* or *TT*.

(a) **Inulin, diodone, chloride and sodium clearances.** The methods for estimating inulin and diodone clearances were those used by Dicker & Heller (1945). The values or T_{Cl} , T_{Na} and T_w (see Dicker, 1946) represented the calculated amounts of Cl^- , Na^+ and water reabsorbed by the tubules, expressed as percentages of the amounts of Cl^- , Na^+ and water filtered through the glomeruli in unit time (Dicker, 1946, 1948*a*).

(b) **Renal response to water administration (water diuresis test).** Rats deprived of food and water for 12 hr. were given by stomach tube an amount of water equal to 5% of their body weight. Urine was collected in graduated cylinders, and the volume excreted recorded at 15 min. intervals. The amount of urine collected was expressed as the percentage of the amount of water administered. Urine collections were carried out for 3 hr.

General procedure. All rats were fed on the standard diet *HH* for 1–2 weeks before being fed on one of the protein-deficient diets (*BB*, *DD*, *FF*, *TT*). Pairs of rats of approximately the same weight were kept in metabolism cages at room temperature. The weight of each animal was recorded daily. The amount of water drunk and that of urine excreted was accurately measured every day. Food was given at 5.00 p.m. each day; the quantities given were 10 g./100 g. animal/day for the diets *HH* and *BB*; and 25 g./100 g. rat/day for the other types of diet. The food not eaten or spilled was weighed the next morning. To permit the comparison between changes in the water metabolism (as assessed by analysis of urine and measurement of the water intake) and changes in blood plasma or tissues, two rats of each series were killed at intervals of 7 days.

Statistical treatment. Results are given as means and standard errors. Fisher's (1944) test for small samples was used for estimating the significance of means. The correlation coefficients (r) were calculated according to Mainland (1938). The probability of P for t and r was obtained from the tables of Fisher & Yates (1943).

RESULTS

Tissue changes during the development of oedema. The mean amount of water drunk by normal rats fed on the standard diet *HH* was 8.4 ± 0.48 ml./100 g./

24 hr.; the metabolic equivalent of the diet amounted to a mean of 6.1 ml. water/100 g./24 hr. Therefore the mean total potential water load amounted to 14.5 ml./100 g./24 hr. About 25% of the total load was excreted by the kidneys (Table 1). When the standard diet *HH* was substituted by the protein-deficient diet *BB*, providing the same number of calories as the former, the amount of water drunk/100 g./24 hr. decreased by 30%, while the metabolic yield of water was 20% more, so that the mean water load was unlikely to have changed significantly (Table 1). The mean urine volume, however, decreased by 50% after a fortnight on the diet *BB*, the kidneys excreting about 14.0% only of the potential water load, instead of 25% as in rats fed on the control diet *HH*.

This decrease of the urine volume occurred independently of changes in the urinary concentrations of Cl^- and Na^+ , which, during that period, remained comparable to those found in animals fed on diet *HH* (Table 1). As a result of the diminished urine volume, the amount of Cl^- and Na^+ excreted decreased by about 50% in a fortnight (Table 1). These findings suggest that when a protein-deficient but isocaloric diet was substituted for the standard diet, there was a diminution in the urine volume which resulted in a Cl^- and Na^+ retention.

When rats were fed on diet *BB* for a longer period (3–4 weeks) the amount of food eaten decreased, and this resulted in a decrease of metabolic water (Table 1). The amount of water drunk was not affected. The urine volume decreased in the same proportion as the potential water load so that the proportion of water excreted did not vary appreciably (Table 1). The urinary concentration of Cl^- and Na^+ , however, fell sharply, Cl^- decreasing by about 55% and Na^+ by about 35%, as compared with their concentrations in control rats. It would thus seem that at this stage of malnutrition, the pronounced retention of Cl and Na ions occurred independently of changes in the water excretion and were the result of changes in the renal activity.

After 5 or 6 weeks there was no significant change in the potential water load, but a bigger proportion of it was excreted by the kidneys; compared with that of the 2 preceding weeks, the increase of the urine volume amounted to about 60%. This increase explained why, in spite of a further fall in the urinary concentration of Cl^- and Na^+ , the mean amount of these ions excreted remained practically unchanged (Table 1).

When correlating these findings with changes in the plasma and muscles, it was found that up to the end of the first fortnight little change occurred in the blood plasma; the concentration of plasma proteins was normal, and changes in the plasma water content were insignificant (Table 1). The early decrease of the urine volume, and hence the initial water re-

Table 1. *Changes of water and ion excretion and of plasma and tissue water content during development of oedema, and during recovery from oedema*

The values are means or means and standard errors. All values for muscle are expressed per 100 g. fat-free tissue.

Type of diet	HH	BB	BB	BB	BB	BB	BB	BB	BB	HH	HH	HH
No. of weeks	...	...	...	...	...	...	...	...	...	...	...	...
Quantity of food eaten/100 g. rat/24 hr.	...	...	...	...	...	...	...	...	...	...	...	...
Metabolic water (ml./100 g./24 hr.)	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0	10.0
Amount of water drunk (ml./100 g./24 hr.)	6.1	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3	7.3
Mean water load (ml./100 g.)	8.4 ± 0.48	6.0 ± 0.14	5.5 ± 0.17	5.1 ± 0.45	5.2 ± 0.25	5.9 ± 0.73	6.0 ± 0.65	9.1 ± 0.90	8.8 ± 0.42	14.9	15.2	14.9
Urine volume:												
ml./100 g./24 hr.	3.6 ± 0.22	2.0 ± 0.15	1.8 ± 0.23	1.3 ± 0.21	1.5 ± 0.23	2.3 ± 0.27	2.2 ± 0.24	2.8 ± 1.23	3.4 ± 0.26	30.7	30.7	30.7
% of water drunk	43.0	33.3	30.0	25.5	28.9	40.0	36.6	30.7	38.6	22.8	22.8	22.8
% of water load	24.5	15.0	14.0	11.8	14.5	25.3	21.1	18.4	22.8	14.9	14.9	14.9
Urine composition:												
Amount of Cl ⁻ present (mg./100 ml.)	366.0 ± 16.31	370.0 ± 19.5	360.0 ± 13.7	242.5 ± 37.30	159.8 ± 18.43	101.2 ± 4.33	95.0 ± 3.42	184.6 ± 50.42	182.0 ± 27.78	182.0 ± 27.78	182.0 ± 27.78	182.0 ± 27.78
Amount of Cl ⁻ excreted (mg./24 hr.)	13.8 ± 1.00	7.4 ± 0.62	6.9 ± 0.86	1.9 ± 0.23	2.5 ± 0.29	2.7 ± 0.31	2.1 ± 0.29	5.2 ± 1.86	6.2 ± 0.19	6.2 ± 0.19	6.2 ± 0.19	6.2 ± 0.19
Amount of Na ⁺ present (mg./100 ml.)	170.0 ± 5.72	—	165.7 ± 18.03	—	112.5 ± 6.04	—	82.3 ± 5.23	142.1 ± 10.87	146.0 ± 14.0	146.0 ± 14.0	146.0 ± 14.0	146.0 ± 14.0
Amount of Na ⁺ excreted (mg./24 hr.)	6.1 ± 0.49	—	3.0 ± 0.57	—	1.7 ± 0.23	—	1.8 ± 0.32	4.0 ± 0.35	5.0 ± 0.23	5.0 ± 0.23	5.0 ± 0.23	5.0 ± 0.23
Amount of K ⁺ present (mg./100 ml.)	940.0 ± 20.02	—	523.3 ± 30.12	—	442.5 ± 84.21	—	—	778.5 ± 38.48	—	—	—	—
Amount of K ⁺ excreted (mg./24 hr.)	32.4 ± 2.14	—	9.4 ± 2.22	—	5.9 ± 0.81	—	—	27.2 ± 3.93	—	—	—	—
Plasma water content (%)	91.4 ± 0.13	91.6 ± 0.10	91.5 ± 0.19	92.0 ± 0.15	92.7 ± 0.21	93.2 ± 0.14	93.1 ± 0.18	92.7 ± 0.15	91.8 ± 0.12	91.8 ± 0.12	91.8 ± 0.12	91.8 ± 0.12
Muscle water (ml./100 g.):												
Total	75.7 ± 0.15	76.0 ± 0.25	76.7 ± 0.12	76.8 ± 0.31	77.0 ± 0.23	77.2 ± 0.21	77.6 ± 0.28	77.0 ± 0.35	76.8 ± 0.30	76.8 ± 0.30	76.8 ± 0.30	76.8 ± 0.30
Extracellular	16.5 ± 0.32	16.6 ± 0.08	18.9 ± 0.17	24.2 ± 1.91	27.8 ± 2.78	35.0 ± 1.24	35.8 ± 1.32	26.0 ± 0.42	18.4 ± 0.17	18.4 ± 0.17	18.4 ± 0.17	18.4 ± 0.17

tention, was manifest in an increase of the muscle water content, which rose from a mean of 75.7–76.7 ml./100 g. fat-free muscle after a fortnight (*t*, 2.786). It was accompanied by a slight but significant increase of the extracellular fluid space of muscles (*t*, 2.891) (Table 1).

These tissue changes occurring at the beginning of the period of malnutrition (1st–2nd week) were small, however, when compared with those observed at the time when the fall in the urinary concentrations of Cl⁻ and Na⁺ occurred (3rd–4th week); the plasma water content rose significantly (*t*, 3.420) and also the muscle water content; this was accompanied by a pronounced increase of the extracellular fluid phase which rose from 18.9 to 27.8 ml./100 g. fat-free tissue; i.e. an increase of nearly 50% (Table 1).

During the 5th–6th week, little change occurred in the water content of plasma and muscle; there was, however, a further increase of the extracellular fluid phase of muscle (Table 1). The increase of the urine volume observed during that period (Table 1) would account for the comparatively small increases of the plasma and muscle water content.

It seemed thus that the development of tissue oedema in rats fed on the protein-deficient diet *BB* proceeded by two different stages: (*a*) the onset of the oedema which coincided with an initial decrease in the urine volume and appeared to be the result of water retention, and (*b*) its full development which occurred when the urinary concentration of Cl⁻ and Na⁺ decreased markedly, i.e. when a significant retention of these ions occurred.

Tissue changes during recovery from nutritional oedema. After having been fed for 6 weeks on the protein-deficient diet *BB*, animals were allowed to recover by being fed on the standard diet *HH* again. When given this high-protein diet the rats ate voraciously. The amount of food allowed was 10.0 g./100 g. rat, yielding approx. 30.0 kg.cal. Though no investigations were made on the motility of the alimentary tract or on its capacity for food absorption, it was noted that, in animals killed after a week, the stomach was not emptied 17 hr. after being fed. It was also found, especially in rats killed after 2–3 weeks of recovery after re-feeding with diet *HH*, that the abdomen was bulging with fat, and that even the muscles had a fatty consistency, findings in striking contrast with those of normal animals.

Table 1 shows that as soon as the high-protein diet (*HH*) was substituted for the protein-deficient diet (*BB*) there was an increase in the amount of water drunk, which rose from a mean of 6.0–9.1 ml./100 g./24 hr. This increased amount of water drunk combined with the metabolic water of the standard diet resulted in a mean potential water load of 15.2 ml./100 g./24 hr., a figure comparable to that observed in control animals (Table 1).

The volume of urine excreted increased but remained still below normal values (Table 1). During the first week of recovery about 18% of the potential water load was excreted by the kidneys, as compared with nearly 25% in normal rats, indicating that during the first days of recovery, the rats retained a significant amount of water.

phase occurred without important changes in the total muscle water content (Table 1). Nor did the plasma water content vary much; it decreased by less than 1%. Such a small change in the plasma water content agreed with the fact that only about 18% of the water load was excreted by the kidneys, as compared with 25% in controls.

Fig. 1. Water diuresis in rats fed on a protein deficient diet (BB): ●—●, normal rats fed on the standard diet HH; [---], same rats fed on diet BB for 14 days; ▲—▲, same rats fed on diet BB for 28 days; ○---○, same rats fed on diet BB for 35 days; △—△, same rats fed on diet BB for 42 days. All animals received an amount of water equal to 5% of their body weight at start of experiment. The vertical lines indicate the standard errors.

The urinary concentration of Cl^- and Na^+ increased by 95 and 73% respectively, but remained significantly below normal values (Table 1). The enhanced urinary concentrations of Cl^- and Na^+ , accompanied by a slight but significant increase of the urine volume, resulted in a pronounced increase in the excretion of these ions. These findings indicated that the rats were losing some of their extracellular fluid. This could be confirmed by estimating the Cl^- and Na^+ space of muscle. During the first week of recovery there was a significant contraction of the extracellular fluid phase: it decreased from a mean value of 35.8–26.0 ml./100 g. fat-free muscle (Table 1). This decrease of the extracellular fluid

During the second week of the recovery process, there was a further increase of the urine volume: it amounted to a mean value of 3.4 ml./100 g./24 hr., a figure comparable with that for normal rats (Table 1). Concurrently the amount of the potential water load excreted by the kidneys rose to about 23%, which is very near to that observed in normal animals.

The urinary concentration of Cl^- and Na^+ , however, remained practically the same as during the first week of recovery. The increase of the Cl^- and Na^+ urinary excretion was thus the result solely of the increased urine volume (Table 1). Plasma water content decreased to nearly normal values: it

amounted to a mean value of $91.8 \pm 0.12\%$, as compared with $91.4 \pm 0.13\%$ in controls (t , 2.015; $P < 0.1$). The muscle-water content, however, was still significantly higher than in normal tissue; in spite of a further pronounced decrease of the muscle extracellular fluid phase, it still amounted to a mean value of 18.4 ± 0.17 ml./100 g. fat-free muscle, which

corresponding to a diminution of the extracellular fluid phase, without marked changes in the total water content of plasma and muscle; later on, the urine volume increased concurrently with a marked loss of the plasma water content. This occurred without further significant increases of the Cl^- and Na^+ urinary concentration.

Fig. 2. Relation between the diuretic response to water administration and the renal excretion of an antidiuretic substance in protein-deficient rats. A. Rats fed on diet BB: $\times$ — $\times$, urine volume excreted in 3 hr., expressed as percentage of the water administered; $\bigcirc$ --- $\bigcirc$, antidiuretic effect on normal rats produced by injecting the urine of rats fed on diet BB, expressed as percentage of the normal diuretic response to water in the same animals. The arrow indicates when the standard diet was substituted by the diet BB. B. Rats fed on diet TT: $\times$ — $\times$, urine volume excreted in 3 hr., expressed as percentage of the water administered; $\bigcirc$ --- $\bigcirc$, antidiuretic effect on normal rats produced by injecting the urine of rats fed on diet TT, expressed as percentage of the normal diuretic response to water in the same animals. The arrow indicates when the standard diet was substituted for the protein-deficient diet TT. (Note that the diuretic response increases and the antidiuretic titre of the urine decreased at a time when the animals were still on a protein-deficient diet.)

is significantly higher than that of normal animals (t , 3.747; $P = 0.02$).

These results would indicate that the recovery from oedema due to protein deficiency proceeded by steps which were almost opposite to those by which tissue oedema developed; recovery started with an increased urinary concentration of Cl^- and Na^+ ,

Water diuresis during the development of oedema. Fig. 1 compares the response to water administration of rats fed on a protein-deficient diet (BB) for varying periods with that observed in the same rats while they were fed on a high-protein diet (HH): it will be seen that in rats fed on diet BB for 2 weeks the rate of urine excretion following water administration was

essentially normal. After 4 weeks on diet *BB*, however, there was a pronounced delay in the onset of the water diuresis and a significant decrease in the amount of urine excreted in 3 hr. (Fig. 1). The diuretic response to water decreased further, and after 6 weeks on diet *BB*, water excretion in 3 hr. amounted to about 42 % of the water administered, instead of about 100 % as in normal animals.

Similar experiments (administration of a volume of water equivalent to 5 % of the body water) were carried out on rats fed on different protein-deficient diets (*DD*, *FF* and *TT*); in all these series of rats the administration of the standard amount of water resulted after a certain time in a delay in the onset of the water diuresis and a decrease of the total amount of urine excreted in 3 hr. These findings confirm those of Dicker *et al.* (1946). The greatest decrease of the renal response to water administration occurred after 6–7 weeks in rats fed on diets *FF* or *TT* (Fig. 2), and after 4 weeks in animals fed on diet *DD*. When rats were fed on a protein-deficient diet for longer periods, it was noted that there was no further decrease in the diuretic response to water administration; on the contrary, in many rats there was an increase in the renal response and a decrease of the delay in the onset of the diuresis, though the urine volume excreted in 3 hr. still remained significantly lower than that of normal rats (Fig. 2). This phenomenon was particularly evident in animals fed on diet *FF* or *TT*, which contained about 75 % of water; it was never observed in animals fed on diet *DD*.

The progressive decrease in the response to water could be correlated with the increase of the Cl^- and Na^+ spaces of fat-free muscles. For instance, in rats fed on diet *DD*, the correlation coefficient (r) amounted to -0.951 , $P < 0.001$ (regression line, $x = -1.90$, $y + 121.4$). Similar results were obtained in rats fed on other protein-deficient diets. In rats fed on diets *BB*, *FF* or *TT*, however, this correlation coefficient was significant for a limited period only (up to 6 or 7 weeks) and ceased to be significant towards the end of the experimental period, i.e. when the renal response to water administration tended to increase.

Water diuresis during recovery from nutritional oedema. When recovering from their hypoproteinaemia as a result of being fed on a high-protein diet (*HH*) again, it could be shown that the diuretic response to water administration became gradually normal (Fig. 2*B*). Towards the end of the first week of recovery, the administration of the standard amount of water produced a diuresis which was significantly greater than that obtained in the same rats when fed on a protein-deficient diet; the delay in the onset of the diuresis was also decreased. However, it was only at the end of the second week of the recovery process that the administration of the standard

amount of water produced a diuresis which was comparable with that observed in normal rats.

These findings would thus indicate that while fed on a protein-deficient diet, rats were unable to excrete the water administered in the same manner as when fed on a high-protein diet. It remained to investigate whether the decreased response to water administration observed in these animals was the result of an impairment of renal functions or whether it was of extra-renal origin, due, for example, to increased retention of water by the tissues.

Renal function tests during the development of oedema. Clearance estimations were made in two series of rats fed on diets *BB* and *TT*: (i) while the tissue oedema developed, and (ii) when the oedema was steadily maintained.

(a) *Inulin clearances* (glomerular filtration rate = G.F.R.). As long as the plasma protein concentration remained within normal limits values for G.F.R. were independent of the water load, and hence of the urine flow (U). The rate of tubular water reabsorption (T_w) was significantly correlated with that of urine flow (Table 2) and changes in urine output were the result of changes in T_w . When the plasma protein concentration started to decrease, G.F.R. and urine flow became correlated (Dicker, 1949*a*), and in rats fed on a protein-deficient diet for 6 to 7 weeks G.F.R. was closely correlated with the urine flow (Table 2) (Dicker *et al.* 1946); at that stage, T_w was found independent of the rate of urine flow (Table 2), i.e. in rats suffering from protein deficiency changes in urine flow were the result of changes of G.F.R.

Very soon after the standard diet had been substituted by a protein-deficient diet, there was a marked decrease of the mean values for G.F.R. (Table 2); at that stage G.F.R., however, was still independent of the urine flow (Table 2). This fall of the mean G.F.R. was accompanied by changes in the rate of tubular water reabsorption (Table 2). In rats fed on the standard diet, the coefficient of correlation (r) between T_w and urine flow was: -0.702 (regression line, $T_w = -98.3 U + 99.3$), while in rats fed for 10 days on diet *TT*, $r = -0.964$ (regression line, $T_w = -212.0 U + 99.5$). It follows from the difference between the slopes of the two regression lines that in rats fed for 10 days on diet *TT*, and having a mean of G.R.F. = $0.48 \text{ ml./100 g./min.}$, comparatively bigger changes in T_w would be necessary to produce changes in the rate of urine flow, than in rats fed on the standard diet.

(b) *Diodone clearances* were estimated only in rats in which the plasma water content was above 93.0 %. The values for Tm_d (maximal rate of tubular excretion for diodone = tubular excretory mass) found were significantly lower than in normal rats (t , 3.929; $P > 0.001$) and agreed with those found previously (Dicker *et al.* 1946).

Table 2. *Inulin clearance estimations and rate of tubular water reabsorption in rats fed on a standard and on a protein-deficient diet*

(G.F.R.=glomerular filtration rate (inulin clearance); T_w =amount of water reabsorbed by the tubules expressed as percentage of the amount of water filtered (G.F.R.); U =urine flow.)

Rats fed on vegetable low-protein diet <i>TT</i>									
Rats fed on standard diet <i>HH</i>			(a) For 10 days			(b) For 42 days			
G.F.R. (ml./100 g./ min.)	Urine flow (ml./100 g./ min.)	T_w (%)	G.F.R. (ml./100 g./ min.)	Urine flow (ml./100 g./ min.)	T_w (%)	G.F.R. (ml./100 g./ min.)	Urine flow (ml./100 g./ min.)	T_w (%)	
0.70	0.0070	99.0	0.52	0.0060	99.2	0.12	0.0045	96.2	
0.72	0.0085	98.7	0.46	0.0080	98.3	0.10	0.0070	93.0	
0.86	0.0092	98.9	0.48	0.0090	98.1	0.15	0.0095	93.7	
0.83	0.0105	98.8	0.55	0.0100	98.1	0.24	0.0120	95.0	
0.90	0.0110	98.8	0.42	0.0105	97.4	0.25	0.0130	95.1	
0.60	0.0130	97.8	0.47	0.0125	97.2	0.30	0.0150	95.0	
0.73	0.0180	97.5	0.49	0.0175	96.3	0.40	0.0200	95.1	
0.78	0.0250	96.8	0.53	0.0250	95.3	0.52	0.0250	95.2	
0.74	0.0305	96.0	0.50	0.0300	94.0	0.60	0.0300	95.0	
0.85	0.0350	95.8	0.42	0.0330	92.1	0.58	0.0322	94.4	
0.69	0.0360	94.8	0.43	0.0380	90.7	0.73	0.0350	95.2	
0.75	0.0480	93.2	0.52	0.0450	91.3	0.80	0.0425	94.5	
Means	0.76	0.0209	97.2	0.48	0.0204	95.2	0.40	0.0205	94.9
S.E.	±0.024	±0.00373	±0.52	±0.011	±0.00371	±0.83	±0.064	±0.00344	±0.20
Correlation coefficient	$r, -0.702; P<0.01$		$r, -0.964; P<0.001$			$r, +0.934; P<0.001$			
Regression line	$T_w, -98.3 U + 99.3$		$T_w, -212.0 U + 99.5$			G.F.R., $+17.2 U + 0.05$			

(c) *Clearances of chloride and sodium ions.* For the first 3–4 weeks, depending on the type of diet fed, clearances of Cl^- and Na^+ remained comparable with those found in normal rats (Dicker, 1948a). In rats fed for 5–6 weeks on diet *TT*, there was a marked decrease in the clearances of Cl^- and Na^+ , with a fall in the calculated values for T_{Cl} and T_{Na} . In rats fed on diet *BB* this decrease of the mean value for Cl^- and Na^+ clearances occurred only after 6–8 weeks. In the two series of rats investigated, the depression of the reabsorptive mechanism for Cl^- and Na^+ and that for the transfer of diodone were found to coincide, and to occur when G.F.R. was most closely correlated with the rate of urine flow.

The antidiuretic activity of the urine of rats fed on a protein-deficient diet. From the results so far presented it will be seen that renal functions of rats fed on a protein-deficient diet showed the following characteristics: (a) soon after the standard diet had been substituted by a protein-deficient diet, there was a decrease in the urine output (Table 1); (b) there was a decreased renal response to water administration (Fig. 1); (c) the slope of the regression line of T_w correlated with urine flow was different from that of rats fed on the standard diet. These findings suggest that the changes in renal function in these rats are due to a marked antidiuretic effect. The urine of normal rats has no antidiuretic activity (Heller & Urban, 1935). The urines of rats fed on diets *BB*, *DD*, *FF* or *TT* were therefore assayed for the presence of an antidiuretic substance.

Normal rats were given an amount of water equal to 5% of their body weight and were injected subcutaneously with 1.0 ml./100 g. of concentrated urine from rats which had been fed on a protein-deficient diet for various periods. Urine from normal rats, even when concentrated up to 50 times, never exerted any antidiuretic effect. In contrast, urine from rats fed on any of the protein-deficient diets used had an unmistakable antidiuretic activity (Fig. 2A, B). In rats fed on diet *BB*, the maximum antidiuretic effect was reached after 7 weeks; in rats fed on *DD* or *TT*, the maximum was reached between the 4th and 5th week. It will be seen, however (Fig. 2A), that when rats were fed on a protein-deficient diet for longer periods, there was a decrease in the antidiuretic titre of their urine. This decrease of the antidiuretic activity of the urine occurred at the same time as the increase of the diuretic response to the administration of the standard amount of water (Fig. 2), i.e. at a time when the mechanism of urinary excretion was regulated by changes in the glomerular filtration rate more than by changes in the tubular activity.

When rats were allowed to recover from their oedema of malnutrition, by being fed on a high-protein diet (*HH*) the antidiuretic activity of their urine decreased progressively; it will be noted, however (Fig. 2B), that even after having been fed on a standard diet *HH* for a fortnight, there was still some antidiuretic substance present in the urine. This finding may be correlated with the fact that in

these animals, when killed, the muscle-water content and the extracellular fluid phase were still significantly higher than in normal rats (Table 1).

It was beyond the scope of this work to investigate the chemical nature of this antidiuretic substance; it was found, however, that in contrast to vasopressin (Harris, 1948) it is not dialysable and that its activity is not significantly destroyed by the addition of an equal volume of 2N-NaOH to the concentrated urine (Dudley, 1920).

DISCUSSION

It would appear from this investigation that when a protein-deficient diet is substituted for a standard diet in rats, there is a sudden decrease of the amount of water cleared by the kidneys. A similar observation had been made in human beings fed on a diet low in protein (Folin, 1905). The decrease of the urine output in rats is not the result of a decrease in the water load of the animals for the following reasons. (a) The rats ate the same quantity (10 g./100 g. rat) of a diet which, though low in its protein content, yielded the same amount of calories and had the same water and salt content as the standard diet (*HH*). The water made available by combustion amounted to 1.06 g. water/g. food for the protein-deficient diet (*BB*) and to 0.94 g. water/g. food for the standard diet (*HH*). Thus, by substituting a high-carbohydrate low-protein diet (*BB*) for a protein one (*HH*), there would be potentially an excess of 1.20 g. water/100 g. rat. (b) Assuming that the amount of 'deposit protein'/kg. body weight was the same in normal rats as in human beings (Boothby, Sandiford, Sandiford & Slosse, 1925), this 'deposit protein' would be held in association with an amount of water equivalent to about 14 % of the body weight; when rats were fed on a protein-deficient diet, this protein reserve would be drawn upon and utilized, thus liberating a significant amount of water, which, added to the surplus of metabolic water from diet *BB*, may explain the decrease of the amount of water drunk, but not the decrease of the urine volume.

The question then was whether the decrease of the urine volume, which occurred almost simultaneously with the change of diet, was produced by changes in the kidney functions, or whether it was the result of water retention by the tissues. From the results of inulin clearance estimations made during the first 2 weeks of feeding on a protein-deficient diet, it would seem that a decrease in the mean glomerular filtration rate accompanied by a change in the rate of tubular water reabsorption (T_w) might account for the decreased urine volume; from the difference in the slopes for T_w values in normal rats and in animals fed on a protein-deficient diet, it can be deduced that a comparable decrease of the urine output involves

a higher rate of tubular water reabsorption in the latter than in the former; e.g. if an initial urine output of 0.050 ml./100 g./min. in both series of rats decreased to 0.025 ml./100 g./min., this would mean that T_w in normal rats increased by 3.5 % (from 93.4 to 96.7 %), while T_w in rats fed on a protein-deficient diet increased by 5.5 % (from 90 to 95 %). In view of the fact that a decrease of the urine output occurred soon after feeding on a protein-deficient diet, in spite of the fact that glomerular filtration rate remained constant, it can be assumed that the initial water retention observed in these rats was produced by an enhanced tubular water reabsorption, as resulting from the action of an antidiuretic substance. This assumption is supported by the fact that, in contrast to normal rats, an antidiuretic substance was actually found in the urine of rats fed on a protein-deficient diet.

Though it was not possible to determine the nature of the antidiuretic substance, the fact that it occurred while the plasma-water content increased makes it unlikely to be a secretion of the posterior pituitary gland. Its presence in the urine of rats fed on a protein-deficient diet would also provide an explanation for the small diuretic effect following water administration to these animals. A decrease of the renal response to the administration of water has been reported by Leslie & Ralli (1947) in rats fed on a diet containing 38 % of fat; they attributed it to the high fat content of the diet and not to its low protein content. This explanation cannot apply to the present series of results, as both the normal diet (*HH*) and the protein-deficient diet (*BB*) contained the same amount of fat, i.e. 12.5 %, while the other diets *DD*, *FF* or *TT* contained even less (3.5 %).

While no evidence could be obtained from clearance tests that renal function was impaired in the early stage of malnutrition, there was good evidence of a renal deficiency at a later period; after several weeks on a protein-deficient diet, when the plasma water content was significantly increased and the urinary excretion seemed to be regulated mainly by changes in glomerular filtration rate, the clearances of diodone, Cl^- and Na^+ were markedly depressed, indicating a severe impairment of tubular excretory and reabsorptive activity.

These findings were reminiscent of those encountered in newborn children or animals: it will be remembered that in newborn children the mean rate of glomerular filtration was found to be much lower than in adults, and in contrast with adults to be correlated with the degree of hydration (Young & McCance, 1942; Dean & McCance, 1948). Tubular function studied by use of diodone or *p*-amino-hippuric acid clearances showed that in newborn children, the tubular excretory mean (T_m) values for either of these substances were considerably depressed (Dean & McCance, 1948), and so were

clearances of Cl^- , Na^+ and K^+ (McCance & Young, 1941). Furthermore, it was shown by Heller (1947) and McCance & Wilkinson (1947) in newborn rats, and Adolph (1943) on young puppies, that the diuretic response to water administration was either very much diminished or even completely absent.

In the present series of rats, all the findings suggested the possibility that the occurrence of tissue oedema was accompanied by a reversal of the adult type of kidney to that of a newborn; whether these changes in the renal function were the result of the low plasma protein concentration encountered in these animals could not be ascertained; in view of the accumulating evidence that no relation between the degree of hypoproteinaemia and the occurrence of oedema of malnutrition can regularly be demonstrated (Stare, 1945; Davidson, Wilcke & Reiner, 1946; Keys *et al.* 1946; Dicker, 1948*b*; Gollan, 1948) it is unlikely that the plasma protein concentration had any direct bearing on the renal functions.

It is of interest, however, to remember that Eggleton, Pappenheimer & Winton (1940) showed on an isolated kidney preparation that the diuresis resulting from a dilution of the plasma protein was mainly due to changes in the glomerular filtration rate, whereas dilution diuresis in an anesthetized dog was mainly the result of a change in the tubular activity involving reduction in the reabsorption of water.

Summing up, there is evidence for attributing the decrease in urine volume which occurred in rats soon after a protein-deficient diet was substituted for a standard diet to an enhanced tubular water reabsorption, the other kidney functions remaining unaffected. At a later stage, however, when tissue oedema was fully developed, renal impairment involving alterations of the tubular mechanisms for the reabsorption of water and ions and the regulation of urine volume by changes in the glomerular filtration rate could be clearly demonstrated. A renal factor operating at the onset and during the maintenance of the oedema can therefore be postulated.

SUMMARY

1. When a protein-deficient isocaloric diet was substituted for a standard diet in rats, there was a sudden decrease of the amount of water excreted by the kidneys. This decrease of the urinary volume could not be explained by a change in the total water load. The urinary concentration of chloride and sodium ions remained unaffected. The urinary ex-

cretion of Cl^- and Na^+ /24 hr. was, however, markedly decreased.

2. After several weeks on a protein-deficient diet, the urinary concentration of Cl^- and Na^+ decreased, while the urine volume increased. In spite of this increase of the urine volume there was a further decrease of the urinary excretion of Cl^- and Na^+ .

3. Correlating these changes in the urinary excretion with those occurring in blood plasma and skeletal muscle in rats fed on a protein-deficient diet it could be shown that: (a) the initial decrease of the urine volume, without changes in the ionic concentration, corresponded to a slight but significant water retention accompanied by a small increase of the extracellular fluid space; (b) the decrease of the urinary concentration of Cl^- and Na^+ coincided with a marked increase of the extracellular fluid phase.

4. Clearance estimations of inulin, diodone, Cl^- and Na^+ showed that: (a) Soon after the rats were put on a protein-deficient diet, the mean glomerular filtration rate decreased, but remained independent of the urine flow; this decrease in the glomerular filtration rate was accompanied by an increase in the tubular rate of water reabsorption. Diodone, Cl^- and Na^+ clearances were normal. (b) After several weeks on a protein-deficient diet, glomerular filtration rate was closely correlated with that of urine flow. The clearances of diodone, Cl^- and Na^+ were significantly depressed.

5. The administration of a standard amount of water by stomach tube to rats fed on a protein-deficient diet resulted in a diuresis which was delayed in its onset and decreased in its amount, when compared with that in normal rats. The first decrease in the renal response to water administration occurred in rats after 1–2 weeks on a protein-deficient diet; the minimum response was observed after about 7 weeks.

6. The urine of rats fed on a protein-deficient diet contained an antidiuretic substance. The antidiuretic activity of the urine increased the longer the animals were fed on one of the experimental diets. The first appearance of antidiuretic activity in the urine coincided with the decreased glomerular filtration rate, the increased tubular water reabsorption and the decreased renal response to water administration. Maximum antidiuretic activity of the urine was found in rats fed for 7–9 weeks on a protein-deficient diet.

7. It is concluded that a renal factor is involved in the onset and maintenance of the oedema of protein deficiency.

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Pigments Derived from Tryptophan: (1) Urorosein, (2) Tryptochrome

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Pigments obtained from tryptophan include representatives of two classes: (1) indigoids, formed by the action of halogens or other oxidizers on free tryptophan; and (2) pigments of the Hopkins-Cole type, formed from free or peptide-linked tryptophan by condensation with aldehydes, and subsequent oxidation. The recently described pigment, tryptochrome (Fearon, 1948) appears to belong to the first class and as indole-3-aldehyde is an oxidation product of tryptophan (Hopkins & Cole, 1903; Ellinger, 1906), a study was made of its chromogenic properties to see if it was an intermediate in the formation of tryptochrome.

UROROSEIN

In presence of acids, indole-3-aldehyde spontaneously changes into the red pigment, urorosein (Ellinger & Flamand, 1909; Homer, 1913, 1915). Urorosein, first obtained by the action of an acid and an oxidizer on urine, is distinguished from other red pigments of urinary origin by: (a) its insolubility in chloroform, (b) its reversible bleaching by alkalis,

and (c) the spectrum of its solution in amyl alcohol, which shows bands at 550–530 and 500–480 m μ . (Maillard, 1913; Homer, 1915). The natural precursor in urine is either 3-indolylacetic acid (Herter, 1908) or the glycine conjugate, *N*-(3-indole-acetyl)-glycine (Ewins & Laidlaw, 1913; Homer, 1913). As yet, no structural formula for urorosein has found general acceptance. Those suggested are: nitroso-indolylacetic acid (Herter, 1908), a triindolyl-methane derivative (Ellinger & Flamand, 1909), and indolylindolylidene (König, 1911). According to Riesser (quoted by Ellinger, 1913), urorosein is an oxidation product of the leuco base,

formed by condensation between 1 molecule of indole-3-aldehyde and 2 molecules of 3-indolylacetic acid. The chemistry of the urinary indigoids, up to 1912, has been reviewed by Maillard (1913), but, as Rimington, Holiday & Jope (1946) observe, there has been much confusion regarding the nomenclature and identity of the red pigments obtainable