SUMMARY

1. The reaction of the sulphur of deaminated wool with alkalis has been studied.
2. It has been found that, excluding the cystine destroyed during deamination, the deaminated wool behaves very similarly to untreated wool with mildly alkaline buffers at 100°; in particular lanthionine is formed.
3. With stronger alkalis at room temperature, however, deaminated wool behaves in an entirely

different manner from untreated wool and no lanthionine is formed.

4. An examination of some of the physical properties of deaminated wool has been made which has in general confirmed earlier observations of Speakman (1936).
5. Difficulties in reconciling the physical properties and the present chemical evidence with current views of wool structure are discussed.

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The Effects of Progressive Nutritional Hypoproteinaemia on the Extracellular-Fluid Phase and Plasma Colloid Osmotic Pressure in Rats

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Many theories have been advanced to explain the mechanism of oedema formation under conditions of malnutrition. It is widely accepted that the main factor concerned is a deficiency of dietary protein, which in turn results in the depletion of the proteins of the body and particularly of those of the blood plasma. According to Starling's (1895) hypothesis a decrease in the plasma proteins results in a fall of the plasma colloid osmotic pressure, a decreased

ability to retain water in the capillaries, and a diffusion of fluid into the tissues. Support for the accepted theory has been provided both by animals fed on a protein-deficient diet (Kohman, 1920; Frisch, Mendel & Peters, 1929; Dicker, Heller & Hewer, 1946) and by human beings suffering from the so-called 'hunger oedema' (Gounelle, Bachet & Marche, 1942; Govaerts & Lequime, 1942; Mollison, 1946). However, Metcoff, Favour & Stare (1945)

claimed that feeding rats with a protein-deficient diet for several days produced no more than a slight decrease of the concentration of plasma proteins, whereas prolonged feeding with a somewhat less protein-deficient diet did not affect the plasma proteins. Furthermore, among clinical cases of 'hunger oedema', reported during and after the last war, a large proportion (seventeen in forty-three in the Govaerts & Lequime (1942) series) had a normal plasma protein level, with a correspondingly high colloid osmotic pressure. Finally, electrophoretic analysis of the plasma proteins of subjects suffering from experimental undernutrition did not show any hypoproteinaemia or a change in the albumin/globulin ratio (Keys, Taylor, Michelsen & Henschel, 1946), even though some of them had developed visible oedema. However, an increase of the thiocyanate space (and therefore very likely of the extracellular-fluid phase) was found in all these subjects whether they had developed oedema or not.

In view of these contradictory findings, the water and electrolyte distribution in skeletal muscle, in liver and in brain were studied in series of rats fed on a low-protein diet. The extracellular- and intracellular-water phases of these tissues were estimated.

METHODS

Experimental animals. Adult male albino rats weighing 270–320 g. were used.

Standard diet (ST). Control rats were given the following diet: casein 18, wheaten starch 29.5, hardened ground-nut oil 9, dried yeast 11, salt mixture (Hawk & Bergeim, 1942) 3, cod-liver oil 2, water 27.5%. Each animal received daily c. 20 g. of this food, representing c. 60 kg.cal.

Vegetable low-protein diet (turnip diet = TT). The diet was modelled on that of Kohman (1920) and of Frisch *et al.* (1929) with fresh turnips taking the place of fresh carrots, and was the same as that used by Dicker *et al.* (1946). It had the following composition: fresh turnips 85.5, wheaten starch 7, hardened ground-nut oil 1.2, salt mixture 4.2, cod-liver oil 2.1%. In addition, each animal received a mixture containing 30 µg. aneurin, 50 µg. riboflavin and 1 mg. nicotinic acid. Fresh turnips contain c. 0.8% of protein.

The supply of this diet was not restricted. It was found that each animal ate c. 50 g. daily, which represented an intake of about 35–40 kg.cal. (The Medical Research Council War Memorandum (1945) was used for the calculation of calorific values.)

Low casein, high carbohydrate diet (LCT). The composition of this diet was as follows: casein 0.5, wheaten starch 54.0, hardened ground-nut oil 7.5%; salt mixture, cod-liver oil, vitamin B and tocopheryl acetate in the same amount as in the TT diet and water 31.7%.

Of this food 100 g. represented 303.5 kg.cal. This diet differed essentially from the TT diet in that the deficiency of calories due to the low protein content was compensated by an increased quantity of starch and fat. Each rat received about 20 g. of this mixture representing a daily intake of 60 kg.cal., comparable with that provided by the ST diet.

All rats had free access to water.

Procedure. All rats were fed on the standard diet for about 4 weeks, before being put on an experimental diet. Series of rats on TT diet were killed after 7, 14 or 42 days. The rats on LCT diet were killed after 70 days. Immediately after death, blood samples were taken and the stomach and the small intestine (up to the caecum) were removed and weighed. The two rectus abdominis muscles and two samples of the liver were excised and the brain removed and cut sagittally. Muscle, liver and brain samples were weighed in weighing bottles and analyzed for their Cl⁻, Na, K and water content.

Analytical data collected. The following were determined for each rat: (a) the content of water, Cl⁻, Na, K and N of heparinized plasma; (b) the content of water, Cl⁻, Na and K of muscle samples; (c) the content of water, Cl⁻, Na and K of liver samples; (d) the content of water, Cl⁻, Na and K of brain samples.

Preparation of muscle, liver and brain tissues for Na and K determination. A known quantity of minced muscle, liver or brain was washed into a digestion tube with 10 ml. of distilled water and allowed to stand overnight. The mixture was then boiled for 20 min. The boiling was repeated twice at intervals of 3 hr. After the last boiling the mixture was allowed to settle, and the supernatant liquid was decanted into a 10 ml. volumetric flask. No filter was used and the device proposed by McCance & Shipp (1933) was adopted. The flask was cooled and its contents made up to the mark. It has been shown by Callow (1929) and McCance & Shipp (1933) that Na and K are quantitatively extracted from muscle tissue by this method. The same method was applied to the extraction of Na and of K from liver and brain samples.

Water determination in plasma and tissues. The water content of plasma, muscle, liver and brain was obtained by drying plasma and tissue samples at 104° for 48 hr.

Estimation of plasma colloid osmotic pressure. Keys & Taylor's (1935a) method and apparatus were used. Duplicate readings were made and corrected to 37°. The suspending and outer buffer fluid was made up as follows: KH₂PO₄ 0.056 mol./l., KOH 0.0395 mol./l. The pH was 7.2.

Defatting of tissues. To express the water content of muscles as ml./100 g. of fat-free tissue, extraction of neutral fat was carried out according to Hastings & Eichelberger (1937). The same technique of fat extraction was applied to brain samples (Manery & Hastings, 1939).

Cl⁻ determination. Cl⁻ in plasma was determined by Whitehorn's (1921) method, using a 0.02N-NH₄CNS solution. For muscle, liver and brain tissue, the titration was carried out after acid digestion (Hastings & Eichelberger, 1937).

Na determination. Na in plasma and in tissue extracts was determined colorimetrically by the method of McCance & Shipp (1931).

K determination. K in plasma and in tissues was determined by Kramer & Tisdall's (1921) method as modified by McCance & Shipp (1933), using the empirical factor 0.12.

Protein determination in plasma. The plasma N content was estimated by a micro-Kjeldahl method. The digestion was carried out with SeO₂, and continued for 5 hr. after the clearing of the solution (Hoch & Marrack, 1945). The N was estimated colorimetrically, after nesslerization, and the protein concentration (in g./100 ml.) was calculated by multiplying the figures for N by 6.25.

The albumin fraction was estimated by the same method, the globulin having been precipitated by sodium sulphite (Campbell & Hanna, 1937).

Estimation of the extracellular-fluid phase of a tissue. The extracellular space of a tissue can be expressed either per 100 g. of fat-free solids or per 100 g. of fat-free wet tissue (Darrow, 1946; Fulton, 1946). The first method gives a better correlation between changes of the fat-free solids and their constituent N, P and K; on the other hand, the relationship between fat-free solids and tissue water, Cl^- and Na is not precise (Darrow, 1946). As changes of water, Cl^- and Na content of tissue were the main object of the present investigations, the second method was deliberately adopted.

The extracellular-fluid phase was expressed in terms of Cl^- (or Na) space. The method of calculating the Cl^- space was that of Eichelberger (1939):

$$\frac{\text{Extracellular water (ml./100 g. tissue)} = \frac{\text{mg. Cl}^-/100 \text{ g. tissue} \times \text{plasma water content}}{\text{mg. Cl}^-/100 \text{ g. plasma} \times 1.04} \times 100.$$

To calculate the Na space a similar formula was used:

$$\frac{\text{Extracellular water (ml./100 g. tissue)} = \frac{\text{mg. Na/100 g. tissue} \times \text{plasma water content}}{\text{mg. Na/100 g. plasma} \times 0.95} \times 100.$$

The cellular phase (g./100 g. tissue) = 100 - extracellular-fluid phase.

Calculation of the intracellular-fluid phase of a tissue. The intracellular-fluid phase was obtained by subtracting the figure for the extracellular-fluid phase from that of the total amount of water in 100 g. tissue.

Statistical treatment. Student's 't' test for small samples, as described by Fisher (1944), was used for estimation of the significance of means. The probability 'P' for 't' was obtained from Fisher & Yates's (1943) tables.

RESULTS

External appearance of rats on protein-deficient diets

Rats fed on *TT* diet for 7 and 14 days looked perfectly healthy, though their weight had decreased by 17 and 23 % respectively. However, rats fed on *TT* diet for 6 weeks looked emaciated. Their loss of

body weight amounted to 33%. Their coats lost the normal smooth appearance, but the skin of the tail and feet was smooth. No ophthalmia or diarrhoea was noticed.

Rats fed on the low protein, high carbohydrate diet (*LCT*) lost 30 % of their weight in 70 days. Nearly all of these animals showed large patches of alopecia, either on the back or on the head. They did not look emaciated.

Gross post-mortem findings in rats fed on protein-deficient diets

The post-mortem findings were as follows: nothing abnormal noted in rats fed on *TT* diet for 7 or 14 days. Oedema of the prepuce was observed in some of the rats which had received it for 6 weeks. No ascites was found in the present series. About two thirds of the animals had 'moist' tissues, while the remainder seemed to be abnormally 'dry'. None of the thoracic or abdominal organs showed any changes; in particular the liver appeared entirely normal. The latter findings conform to those made with a previous series of *TT* rats (Dicker *et al.* 1946). No ascites was found in *LCT* rats. All these animals had fatty deposits in the abdomen; their livers had a markedly fatty appearance.

Changes in plasma water concentration

There was no significant decrease in the plasma solids of rats fed on *TT* diet for 7 days as compared with control rats ($t=1.312$; $P=0.2$), nor was there any significant change in the protein concentration of their plasma (Table 1). But after 2 weeks on *TT* diet there was a marked increase in the plasma water accompanied by a decrease in the plasma protein concentration. Expressed in terms of plasma solids, there was a fall of 15%, while the protein level fell by 10 % (Table 1).

The dilution of the plasma became more apparent the longer the period of malnutrition: after 6 weeks on *TT* diet the fall in the plasma solids and in the

Table 1. *Effect of protein-deficient diets on the total water content of plasma, muscle, liver and brain of rats*

(The values are means and standard errors. In parentheses, number of rats. All values for muscle and brain are expressed per 100 g. fat-free tissue.)

Treatment of rats	Plasma protein concentration (g./100 ml.)	Plasma water content (g./100 ml.)	Muscle water content (g./100 g.)	Liver water content (g./100 g.)	Brain water content (g./100 g.)
Fed on a standard diet (<i>ST</i>)	7.06 ± 0.090 (27)	91.4 ± 0.13 (27)	75.7 ± 0.32 (27)	71.4 ± 0.31 (27)	83.6 ± 0.24 (11)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	6.75 ± 0.331 (9)	91.8 ± 0.15 (9)	75.0 ± 0.51 (9)	72.6 ± 0.48 (9)	82.7 ± 0.21 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	6.46 ± 0.213 (9)	92.7 ± 0.21 (9)	77.0 ± 0.23 (9)	72.3 ± 0.75 (9)	82.2 ± 0.12 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	5.02 ± 0.137 (29)	93.2 ± 0.16 (29)	77.2 ± 0.28 (30)	74.9 ± 0.39 (30)	83.3 ± 0.41 (12)
Fed for 70 days on a low casein diet (<i>LCT</i>)	4.91 ± 0.186 (6)	93.6 ± 0.16 (6)	74.2 ± 0.55 (6)	68.3 ± 1.70 (6)	82.9 ± 0.70 (6)

concentration of proteins was 21 and 27 % respectively (Table 1).

The same phenomenon was noticed in rats fed on a low casein diet (*LCT*) for a longer period. In spite of the gross accumulation of fat in the body, plasma solids and proteins fell by nearly 30 %.

Changes in the water content of tissues

No changes in the total water content of muscles were found in animals which had been fed on *TT* diet for 7 days as compared with control rats ($t=1.136$, $P>0.2$) (Table 1). However, the liver already showed a slight increase of its total water content ($t=2.010$, $P<0.1$).

Table 1 shows changes in total water content of muscles, liver and brain in rats fed on *TT* diet for 14 and 42 days.

When comparing total water content of muscles and liver of rats after 42 days of *TT* diet and after 70 days on *LCT* diet, it will be seen that the latter animals had significantly less water in their tissues than the former ($t=4.411$, $P<0.001$, and $t=5.500$, $P<0.001$); the water content of muscle and liver samples from *LCT* rats was actually comparable with that of control rats.

Changes in the ionic composition of plasma and tissues

Plasma. Changes in the chloride, sodium and potassium concentration of plasma in *TT* rats during varying periods of observation are shown in

Table 2. Comparing these changes with those of the plasma water (Table 1) in the same rats at corresponding periods, it will be seen that changes in the ionic pattern are independent of those of plasma water content, i.e. occasional decreases in plasma sodium (after 7 days) or chloride concentration (after 14 days) are not the result of a dilution of the plasma.

The independence of the concentration of the ions in plasma from that of the plasma water emerges even more clearly when the changes in the potassium level of the plasma are considered: the potassium level in the plasma of *TT* rats rose during the whole period of observation, in spite of the rising dilution of the plasma. In the *LCT* rats, it remained unaffected.

Tissues. It would be tempting to assume that the fact that chloride and sodium were not diluted by an increased amount of water in plasma was the result of an influx of these ions from the tissues. However, Table 2 shows that so far as chloride and sodium are concerned, their concentration increased significantly in muscle and in liver ($t=7.529$, $P<0.001$ and $t=3.231$, $P<0.001$). This was already apparent in rats fed on a turnip diet for 7 days only.

Comparing Tables 1 and 2, it will be seen that, as in plasma, changes in the chloride and sodium concentration of muscle and liver were independent of the concurrent changes in their water content. But in contrast to that of muscle and liver tissues, the

Table 2. *Ionic composition of plasma, muscle, liver and brain in normal and hypoproteinaemic rats*

(The values are means and standard errors. In parentheses, number of rats. All values for muscle and brain are expressed per 100 g. fat-free tissue.)

Treatment of rats	Cl ⁻	Na	K	Cl ⁻	Na	K
	(m-equiv./l. water)	(m-equiv./l. water)	(m-equiv./l. water)	(m-equiv./l. water)	(m-equiv./l. water)	(m-equiv./l. water)
	Plasma			Muscle		
Fed on a standard diet (<i>ST</i>)	83.2±2.34 (27)	128.3±7.05 (18)	5.6±0.29 (17)	20.2±0.47 (17)	28.8±1.48 (17)	126.2±7.73 (17)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	84.6±3.08 (9)	119.3±7.53 (9)	5.0±0.27 (9)	27.8±0.82 (9)	35.2±3.59 (9)	107.6±5.78 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	83.7±4.90 (9)	124.0±6.94 (9)	6.0±0.25 (9)	31.5±1.01 (9)	46.5±1.42 (9)	112.4±7.14 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	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)
Fed for 70 days on a low casein diet (<i>LCT</i>)	81.0±2.03 (6)	120.6±2.46 (6)	5.1±0.20 (6)	37.2±2.09 (6)	60.0±4.60 (6)	107.9±5.30 (6)
	Liver			Brain		
Fed on a standard diet (<i>ST</i>)	33.6±2.80 (11)	31.0±2.09 (10)	112.8±7.63 (10)	42.7±2.89 (9)	49.0±3.56 (10)	124.0±10.51 (10)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	41.4±2.99 (9)	50.7±5.61 (9)	103.7±4.87 (9)	41.1±2.69 (9)	52.3±4.37 (9)	129.5±5.26 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	40.2±0.66 (9)	60.6±3.00 (9)	97.8±8.13 (9)	38.0±1.75 (9)	56.5±2.42 (9)	118.6±3.08 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	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)
Fed for 70 days on a low casein diet (<i>LCT</i>)	49.0±2.69 (6)	57.2±6.21 (6)	120.0±6.63 (6)	41.7±1.25 (6)	58.4±3.36 (6)	109.7±8.89 (6)

ionic composition of brain samples remained constant during the whole period of observation (Table 2).

Such changes in the water content and in the ionic composition of plasma and tissues made it likely that variations had occurred in the amount of extracellular water in muscle and liver.

Extracellular-fluid phase of tissues

In normal rats fed on a standard diet (*ST*) the chloride space of muscle, liver or brain was comparable with, and almost equal to, the sodium space (Table 3). It will also be seen from Table 3 that the extracellular-fluid phase was of characteristic magnitude in each of the tissues investigated and showed little variation in individual normal animals.

However, in *TT* rats, the extracellular-fluid phase of muscle and liver was increased. This increase was already significant after 7 days: it amounted to 44 % in muscles and to 50 % in liver. It increased steadily. The increase after 6 weeks amounted to 163 and 55% of the normal.

In Table 3 all data obtained for rats kept on *TT* diet for 42 days were pooled, irrespective of the fact that about one third of these animals had particularly dry and thin muscles. But the individual figures obtained from analysis of muscle samples of such 'dry' animals showed a marked increase in the extracellular-fluid phase, amounting to nearly 140%. Whether 'dry' or 'wet', all the *TT* rats had therefore a marked 'oedema' of their tissues. It follows that there was no essential difference between the two types of protein-deficient animals (Dicker, 1947).

In rats fed on a low casein, high carbohydrate diet (*LCT*) the extracellular-fluid phase of muscle and liver was also increased. The increase amounted to 120 and 70 % respectively. It is thus clear that the increased extracellular-fluid phase of *TT* and *LCT* rats was not the result of a deficiency of calories.

It could thus be shown that there were signs of early oedema in the tissues of rats fed on a protein-deficient diet. This increase of the extracellular-fluid space developed in spite of normal plasma protein

Table 3. *Extracellular- and intracellular-fluid phases (chloride and sodium) of tissues of normal and hypoproteinaemic rats*

(The values are means and standard errors. In parentheses, number of rats. All values for muscle and brain are expressed per 100 g. fat-free tissue.)

Treatment of rats	Extracellular-fluid phase (ml./100 g.)		Muscle	Intracellular-fluid phase (ml./100 g.)	
	Cl ⁻	Na		Cl ⁻	Na
Fed on a standard diet (<i>ST</i>)	16.7±0.50 (27)	16.8±1.03 (17)		59.0±0.50 (27)	58.8±1.11 (17)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	24.1±1.54 (9)	28.1±3.68 (9)		50.8±1.74 (9)	46.9±3.94 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	32.3±2.78 (9)	30.1±1.89 (9)		44.7±2.72 (9)	47.0±1.83 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	44.8±1.90 (29)	43.5±2.08 (11)		32.4±1.64 (29)	33.6±0.70 (11)
Fed for 70 days on a low casein diet (<i>LCT</i>)	36.9±3.63 (6)	36.8±2.01 (6)		37.3±3.65 (6)	37.4±2.27 (6)
			Liver		
Fed on a standard diet (<i>ST</i>)	22.6±2.50 (11)	21.3±1.55 (10)		48.5±2.05 (11)	50.1±2.03 (10)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	34.3±2.64 (9)	35.2±2.23 (9)		38.6±2.97 (9)	37.6±2.94 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	38.1±2.56 (9)	34.8±2.73 (9)		34.2±2.57 (9)	48.0±2.74 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	35.4±2.66 (11)	32.6±1.91 (11)		39.5±2.52 (11)	42.3±2.14 (11)
Fed for 70 days on a low casein diet (<i>LCT</i>)	39.0±2.03 (6)	35.4±3.22 (6)		29.3±2.07 (6)	32.8±4.09 (6)
			Brain		
Fed on a standard diet (<i>ST</i>)	38.6±3.31 (9)	40.1±3.00 (10)		44.9±3.24 (9)	43.5±2.89 (10)
Fed for 7 days on a protein-deficient diet (<i>TT</i>)	38.7±2.62 (9)	43.0±4.82 (9)		43.2±2.81 (9)	39.5±4.90 (9)
Fed for 14 days on a protein-deficient diet (<i>TT</i>)	40.6±2.24 (9)	38.1±2.24 (9)		41.8±2.18 (9)	44.0±2.29 (9)
Fed for 42 days on a protein-deficient diet (<i>TT</i>)	40.4±3.31 (11)	43.9±2.71 (11)		42.9±3.60 (11)	39.4±2.37 (11)
Fed for 70 days on a low casein diet (<i>LCT</i>)	41.0±1.51 (6)	38.4±2.14 (6)		41.9±1.40 (6)	44.9±2.31 (6)

concentrations. It could be shown further that the extracellular-fluid space of tissues (muscle and liver) increased whether the protein-deficient diet was, or was not, lacking in calories.

The intracellular-fluid phase of tissues

Can the increased extracellular-fluid phase observed in the different series of *TT* and *LCT* rats be explained by changes in the total muscle water? Comparing the data of Table 1 and Table 3, it could be concluded that this was not the case: in animals fed on *TT* diet for 7 days the extracellular-fluid space increased without any changes in the total muscle water. Even in rats fed on *TT* diet for 14 or 42 days the increase in the total water of the tissues (Table 1) was far less pronounced than that of the extracellular-fluid space (Table 3). It follows that tissue oedema developed concurrently with a marked loss of intracellular water. This is even clearer with *LCT* rats (Table 3).

It is thus clear that the retention of tissue water resulting from a lack of protein in the food did not produce a swelling of the tissue cells; on the contrary, protein deficiency led to a decrease of intracellular

water. This decrease became the more obvious the more the extracellular-fluid space increased.

Colloid osmotic pressure of plasma

It has not been shown so far whether the decreases in the plasma protein concentration observed led to corresponding decreases in plasma colloid osmotic pressure. Values of 18.9 ± 0.12 cm. water, corrected to a temperature of 37° , were obtained for normal rat plasma. These values were somewhat lower than those found by Landis (1930), but this difference may be accounted for by the difference in pH and in electrolyte concentration of the buffer solution producing different Donnan effects (Marrack & Hewitt, 1927; Keys & Taylor, 1935a).

Table 4 gives figures for the colloid osmotic pressure of plasma samples from normal and hypoproteinaemic rats.

Dilutions of plasma of normal rats were made by adding varying amounts of the buffer solution. Fig. 1 shows that the colloid osmotic pressure of progressive dilutions of plasma of normal rats yielded values which form a straight line when plotted against the plasma protein concentration.

Table 4. *Colloid osmotic pressure of plasma samples from normal and hypoproteinaemic rats*

	Plasma proteins (Kjeldahl) (g./100 ml.)	Albumin/globulin ratio (A/G)	Plasma colloid osmotic pressure (cm. water)
Normal rats	7.48	1.20	19.8
	7.48	1.10	19.6
	7.25	1.20	20.0
	7.04	1.17	19.4
	6.75	1.07	19.2
Mean and s.e.	7.20 ± 0.100	1.15 ± 0.023	19.6 ± 0.12
Rats fed on a protein-deficient diet (<i>TT</i>) for 7 days	7.18	0.98	19.1
	7.08	1.00	19.6
	7.00	1.26	19.4
	6.87	0.95	18.5
Mean and s.e.	7.03 ± 0.057	1.05 ± 0.031	19.2 ± 0.19
Rats fed on a protein-deficient diet (<i>TT</i>) for 14 days	6.63	0.83	17.7
	6.55	0.90	17.3
	6.08	0.80	16.3
	6.07	0.70	15.4
	6.00	0.70	14.6
Mean and s.e.	6.22 ± 0.121	0.78 ± 0.011	16.3 ± 0.51
Rats fed on a protein-deficient diet (<i>TT</i>) for 42 days	5.94	0.60	12.8
	5.94	0.60	10.8
	5.45	0.68	13.7
	5.27	0.50	8.6
	5.16	0.60	10.0
	5.10	0.62	11.7
	4.52	0.50	5.5
	3.95	0.50	8.6
Mean and s.e.	5.16 ± 0.223	0.57 ± 0.007	10.2 ± 0.87
Rats fed on a protein-deficient diet (<i>LCT</i>) for 70 days	5.14	0.56	10.6
	4.37	0.67	10.8
	4.37	0.68	12.7
	3.91	0.68	11.3
	3.86	0.59	7.8
Mean and s.e.	4.33 ± 0.205	0.63 ± 0.023	10.6 ± 0.71

This relationship applied only for dilutions of the plasma up to 50% of the initial concentration, confirming Verney's (1926) findings on human plasma.

Fig. 1. Relation between the plasma colloid osmotic pressure and plasma protein concentration. $\times$ = normal rats; $\circ$ = rats fed for 7 days on a vegetable protein-deficient diet; Δ = rats fed for 14 days on a vegetable protein-deficient diet; $+$ = rats fed for 42 days on a vegetable protein-deficient diet; $\blacksquare$ = rats fed for 70 days on a low casein diet. All the values for colloid osmotic pressure are corrected to 37°.

Comparing the values for the colloid osmotic pressure of diluted normal plasmas with those of samples from hypoproteinaemic rats, it will be seen (Fig. 1) that, at the same concentration of plasma proteins, the colloid osmotic pressure of plasma of rats fed on *TT* diet for 42 days and of *LCT* rats was lower than that of the normal animals. This decrease in the colloid osmotic pressure per g. plasma protein in animals fed on a low protein diet was progressive: plasmas of rats fed on *TT* diet for 7 days did not show a significant fall in the colloid osmotic pressure ($t = 1.581$, $P > 0.1$), but after 14 days the fall of colloid osmotic pressure was greater than that produced by a corresponding dilution of plasma of normal rats. It is thus clear that the low figures obtained for the osmotic pressure of plasma proteins in rats fed on protein-deficient diets could not be the result of a simple dilution of the blood of these animals.

The decrease in the colloid osmotic pressure observed in the plasma of rats fed on protein-deficient diets was closely correlated with the fall in the concentration of albumin; that of the globulin

remained more or less unaffected. In other words, the colloid osmotic pressure per g. of total plasma proteins was directly proportional to the albumin/globulin (A/G) ratio (Table 4). However, in a few cases plasma samples with the same concentration of proteins had a widely different colloid osmotic pressure, e.g. two plasma samples with protein concentrations of 5.9 g./100 ml. gave values for the colloid osmotic pressure of 12.8 and 10.8 cm. water. The difference in these instances could not be accounted for by changes in the albumin/globulin ratio (A/G = 0.60 in both cases). Two other samples with the same A/G ratio (0.50) had a colloid osmotic pressure of 8.6 and 5.5 cm. water respectively. It is thus clear that in these particular cases, changes in the A/G ratio did not account for the low colloid osmotic pressure. It thus seems likely that the plasma proteins of some of the hypoproteinaemic animals underwent other changes in chemical or physical composition besides the decrease in the albumin fraction.

Finally, is there a relation between the colloid osmotic pressure of plasma and the occurrence of tissue oedema? Comparing the figures in Table 3 and Table 4 it will be seen that the increase in the extracellular-fluid space in rats fed on *TT* diet for 7 days occurred in animals with normal plasma protein concentration, normal A/G ratio and normal colloid osmotic pressure. Furthermore, no correlation could be found between the size of the extracellular-fluid space and the plasma colloid osmotic pressure.

DISCUSSION

There is ample evidence that chloride and sodium in rat and dog muscle are mainly extracellular (Fenn, 1936; Harrison, Darrow & Yannet, 1936; Hastings & Eichelberger, 1937; Manery & Bale, 1941). Further proof for the validity of this statement comes from the work of Manery & Haege (1941), Hevesy & Rebbe (1940) and Cuthbertson & Greenberg (1945) with radioactive isotopes ^{36}Cl and ^{24}Na (or ^{22}Na). These authors have shown that these isotopes were extracellular and that their distribution was the same. Finally, there is a satisfactory agreement between values for chloride space in muscle and liver and the extracellular space as determined microscopically in frozen sections of these tissues (Höber, 1947; Truax, 1939). At the same time it must be remembered that chloride and sodium spaces are likely to be somewhat larger than the true extracellular-fluid space, even in normal animals (Fulton, 1946), and that the possibility obtains that pathological conditions may produce further alteration of cell permeability. The chloride and sodium spaces may, therefore, not be quite identical with the true extracellular space. The term extracellular-fluid space in this paper must be understood to refer to

the apparent volume distribution of chloride and sodium.

In the present investigation it could be shown that, for muscles of normal rats, there is good agreement between the values of the extracellular-fluid phase, whether expressed in terms of chloride or of sodium space ($t=0.113$, $P>0.9$). The values compare favourably with those found by means of radioactive isotopes in the muscle of rats (Cuthbertson & Greenberg, 1945) and in dogs (Eichelberger, 1941). In the liver also it could be shown that the values for extracellular-fluid phase, whether expressed as chloride or as sodium space, were comparable ($t=0.429$, $P>0.6$). They were of the same magnitude as that of chloride space of liver found in dogs (Eichelberger, 1941).

When defining the intracellular-fluid phase it must be remembered that the amount of water inside the cell is correlated with the amount of potassium and that, under normal conditions, there is no shift of bases across the cell membrane (Peters, 1944). There is a reciprocal relationship between the extracellular sodium and the intracellular potassium; in other words, the amount of intracellular potassium, and intracellular water, is indirectly influenced by the concentration of extracellular sodium (Darrow, 1945).

It could be shown in the present investigation that the extracellular-fluid phase of muscle of normal adult rats was fairly constant. However, in rats fed on a protein-deficient diet for a short period only (7 days) the extracellular-fluid space of muscle increased significantly. This onset of tissue oedema occurred without significant changes in the plasma water content, the plasma protein concentration or the osmotic pressure of the plasma proteins. These findings agree with those of Keys *et al.* (1946) in human beings, receiving experimentally a low-protein diet, in whom an increase of the thiocyanate space (i.e. of the extracellular space) could be demonstrated at a time when the plasma protein concentrations, the plasma electrophoretic pattern and the plasma colloid osmotic pressure were still normal. It thus seems difficult to attribute the early onset of the oedema to a fall of the colloid osmotic pressure (Starling's hypothesis), but it might still be due to an increase in the capillary pressure (Pappenheimer & Soto-Riviera, 1947). It was not possible to estimate the venous pressure in the present series of rats; but in human beings Keys *et al.* (1946) showed that the venous blood pressure had fallen by nearly 50% of the normal value.

The cause of the early increase in the extracellular-fluid phase must thus be sought elsewhere. The rats fed on a vegetable low-protein diet were lacking in both proteins and calories. There are thus good reasons to assume that a cellular breakdown was accelerated. Hence the early tissue oedema may

have been due to an accumulation of waste products: if of small molecular weight they would exert a high osmotic pressure. The same process may also involve liberation of potassium ions, which in normal tissue were osmotically inactive. A higher osmotic pressure within the cell would result in an increased concentration of chloride and sodium in the extracellular-fluid space in an attempt to equalize osmotic pressure. To be able to maintain a balance between forces engaged in such a dynamic equilibrium the cells would extrude their waste products and with them part of their potassium, and ultimately would lose some of the intracellular water. This assumption would agree with the findings of Gamble, Ross & Tisdall (1923), who showed that starvation induces a loss of potassium from the body corresponding to the potassium content of the muscle tissue lost.

The data in Table 3 show that the increase of the extracellular-fluid phase was significantly greater than the decrease of the intracellular water. This can only mean that the increased extracellular-fluid phase was made up mainly by water from the plasma. After 7 days on a low protein, low calorie diet (*TT*) the plasma water content and its protein concentration were normal (Table 1). This is in agreement with findings in dogs fasting for a period of 4-6 days (Kerr, Hurwitz & Whipple, 1918). The unchanged level of the plasma proteins in rats fed for 7 days on *TT* diet might be explained by changes in the plasma volume masking the loss of plasma proteins, resulting from a state of semi-starvation. It would also agree with the findings of Metcalf *et al.* (1945) in rats kept on a protein-deficient diet.

At a later stage, in rats fed on *TT* diet for 14 days, the concentration of plasma proteins was decreased, though the A/G ratio was not significantly altered; and the colloid osmotic pressure was somewhat lower than in controls (Table 4; Fig. 1). Though small, these changes in the colloid osmotic pressure of plasma may have been sufficient to produce an increase of the extracellular-fluid phase, provided that there was no concurrent drop in the capillary pressure.

In rats fed on *TT* or *LCT* diet for several weeks the colloid osmotic pressure of the plasma proteins was markedly lower than that of the controls. In some cases it was even lower than that expected for the corresponding amount of plasma proteins. Such discrepancies between the plasma protein concentration and the expected colloid osmotic pressure have been reported in patients suffering from malnutrition (Govaerts & Lequime, 1942) and in young men exercised to exhaustion where, in spite of a marked increase (averaging 12.5%) in serum protein concentration, the colloid osmotic pressure decreased (Keys & Taylor, 1935*b*). In rats fed on a protein-deficient diet (*TT* or *LCT*) the globulin fraction did not seem to be seriously affected; it is thus likely that the abnormally low colloid osmotic

pressure encountered in some cases was the result of changes in the albumin fraction. Two explanations of this phenomenon may be proposed:

(a) The chemical analysis of plasma proteins after precipitation of globulins by sodium sulphite may have given too high values for the albumin fraction (Cohn & Wolfson, 1947). This hypothesis receives further support from the work of Zeldis, Alling, McCoord & Kulka (1945). Comparing data obtained by chemical analysis and by electrophoretic methods these authors have shown that in dogs fed on a protein-deficient diet for 12 weeks the degree of depletion of 'electrophoretic albumin' is considerably greater than that of the chemical albumin fraction.

(b) Changes in the molecular dimension of the proteins or in their ionization, or in both, provide the basis for another interpretation. For any change in the colloid osmotic pressure to be due to changes other than in the molecular dimension, there must be changes in the ionization of the proteins or changes in the concentration of the crystalloids of the medium (Keys & Taylor, 1935*b*). Both pH and ionic strength, inside and outside the collodion membrane of the osmometer, remained constant in the present experiments. This makes any appreciable change in the magnitude of the Donnan effect unlikely. Furthermore, it has been shown that the colloid osmotic pressure of blood plasma is little affected by changes in pH (Marrack & Hewitt, 1927) or by moderate changes in salt concentration (Meyer, 1932).

It would seem that there are two stages in the production of oedema in tissue resulting from feeding rats on a protein-deficient diet. It could be shown that a significant increase of the extracellular-fluid phase of muscles occurred very early, without any significant changes in the water content or the protein concentration of plasma; no changes in the colloid osmotic pressure of plasma were observed at this stage. It must therefore be concluded that the onset of oedema formation in malnutrition is independent of changes in the colloid osmotic pressure of plasma. At a later stage further increases of the extracellular-fluid phase coincided with the progressive fall in the colloid osmotic pressure of the plasma. However, even then no clear correlation could be found between the degree of hypoproteinaemia, the fall of the colloid osmotic pressure of plasma and the magnitude of the extracellular-fluid phase of tissues.

SUMMARY

1. The total water content of plasma, of skeletal muscle, of liver and of brain was estimated in normal rats and in rats fed for periods of 7, 14 and 42 days on a protein-deficient diet. Extracellular- and intracellular-fluid phases of these tissues were calculated by means of chloride and sodium estimations.

2. The calculated values for the extracellular-fluid phase of these tissues agreed well, whether expressed as chloride or as sodium space.

3. In normal rats fed on a standard diet (*ST*) the extracellular-fluid phase of muscle, liver and brain, expressed as chloride space, amounted to 16.7 ± 0.50 ; 22.6 ± 2.50 and 38.6 ± 3.31 ml./100 g. fat-free tissue respectively.

4. In rats fed for 7 days on a vegetable protein-deficient diet (*TT*) the water content of plasma and of tissues, the plasma protein concentration and the plasma colloid osmotic pressure remained unchanged. In spite of this the extracellular-fluid phase of skeletal muscles and of liver had significantly increased, showing that there was tissue oedema with normal plasma protein concentration and normal colloid osmotic pressure.

5. In rats fed for 14 days on a vegetable protein-deficient diet (*TT*) there was a significant increase in the water content of the plasma, the muscles and the liver, with a decrease in the plasma protein concentration and a slight but significant decrease in the colloid osmotic pressure of plasma. The extracellular-fluid phase of the muscles was significantly greater than that of rats fed for 7 days on *TT* diet.

6. Comparing data obtained for rats fed for 42 days on a low-protein low-calorie diet (*TT*) with those for animals fed for 70 days on a diet equally low in protein but yielding an adequate supply of calories (*LCT*) it could be shown that the extracellular-fluid phases of muscle and liver were markedly increased, and the concentration of proteins in plasma significantly decreased in both series.

7. The mean colloid osmotic pressure of normal rat plasma amounted at 37° to 19.6 ± 0.12 cm. water; that of rats fed on *TT* diet for 7 days was 19.2 ± 0.19 cm., that of rats fed on *TT* diet for 14 days was 16.3 ± 0.51 cm., that of animals fed on *TT* diet for 42 days was 10.2 ± 0.87 cm., and that of animals fed on *LCT* diet for 70 days amounted to 10.6 ± 0.71 cm. water.

8. The colloid osmotic pressure of plasma of the three latter groups was correlated with the albumin/globulin (A/G) ratio of plasma, but not with the nitrogen concentration of the plasma samples.

9. In the cases of a few rats fed for 42 days on *TT* diet and for 70 days on *LCT* diet, the colloid osmotic pressure of plasma samples was lower than that expected from either the A/G ratio or the plasma nitrogen concentration.

10. No correlation could be found between the increase of the extracellular-fluid phase of tissues and the decrease of the colloid osmotic pressure of the plasma.

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

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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.