The effect of diuretics, cortisone and prednisolone on weanling rats with oedema produced by an African plantain diet

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Oedema is a constant feature in kwashiorkor. On treatment with a high-protein diet it usually disappears but it disappears slowly: it seems to be closely correlated with the concurrent hypoalbuminaemia (Davies, 1952; Kerpel-Fronius & Varga, 1953), and de Maeyer (1955) has shown that, even in infants which respond favourably to treatment with protein, it takes about 5 weeks before the plasma albumin reaches a normal level. Diuretics have only been used rarely in kwashiorkor (Russell, 1946; Waterlow, 1948; Delon, 1950) and apparently have often been found ineffective, though Waterlow (1948) remarks that 'diuretic therapy may have been life-saving' in some of his cases. All the diuretics used were mercury compounds. It seems indicated to try other diuretic drugs and certain corticoids, since the latter and corticotrophin are known to be effective in nephrotic oedema, i.e. in another condition characterized by low plasma-albumin concentrations.

However, before conducting clinical trials, it was decided to try to produce a condition similar to kwashiorkor experimentally in rats and—if successful—to evaluate various diuretics in such animals. The highest incidence of kwashiorkor is in infants aged 1–3 years. Weanling rats were therefore used which were fed on a diet of boiled plantains (*Musa paradisiaca*) imported from Uganda. The protein content of plantains is low (about 1·0 %) and the ratio of carbohydrate to protein is high. This experimental diet was chosen because inquiries amongst mothers of children with kwashiorkor admitted to Mulago Hospital, Kampala, had shown (Heller & Schnieden, 1955) that in many parts of Uganda boiled plantains form the staple and indeed almost the only food of infants after weaning. Similar diets have been reported by Brock & Autret (1952) from the Belgian Congo and Nigeria. Since climatic factors may influence the metabolism of water and posterior-pituitary function (Macfarlane & Robinson, 1957; Heller, Herdan & Zaidi, 1957) the rats were kept in a psychrometric room at the mean maximum temperature (26·7°) and mean relative humidity (75 %) of Kampala and exposed to light for 12 h daily.

The first part of this paper describes the syndrome produced in the rats fed on the protein-deficient plantain diet, the second shows the effects of the diuretics used on healthy rats at ambient temperatures and humidities, and the third gives the results of the treatment of animals with oedema of protein deficiency.

EXPERIMENTAL

Animals and diet. Three hundred and ninety-five male albino rats (Wistar) weighing initially 35–65 g were used for the experiment at 'tropical' temperature and 325 rats of somewhat greater weight (74–96 g) for the 'pilot' experiments with diuretics. Throughout the investigation litter-mates were used as controls. At the conclusion of between 25 and 32 days on experimental and control diets the rats were killed by decapitation. Diets and water were given unrestricted. The control diet (Heller & Blackmore, 1953) contained: protein 22·5, fat 2·9, carbohydrate 44·4, NaCl 0·3 and moisture 9·2%; the carbohydrate:protein ratio was 2·0. It provided about 295 Cal./100 g. The protein-deficient diet consisted of boiled plantains (the form eaten in Uganda) and contained (means of two samples): protein 1·1, fat 0·2, carbohydrate 18·5 and moisture 70·5%; the carbohydrate:protein ratio was 16·8. It supplied approximately 74 Cal./100 g. The sodium content of the plantains was very low (3·7 m-equiv. Na/100 g boiled plantain). Sodium chloride in the same amounts as present in the control diet was therefore added in some of the experimental series. The potassium content of the plantain diet was 88·5 m-equiv./100 g and that of the control diet 460 m-equiv./100 g. A sample of plantain diet was sent to Dr F. E. Byron (Applied Nutrition Unit, London School of Hygiene and Tropical Medicine) for estimation of lipotropic factors. He reported that it contained 12 mg methionine, 0·42 mg choline and 45 mg inositol/100 g.

Food intake was estimated by placing rats singly into small cages with wire-mesh floors which were suspended above trays lined with filter-paper. Weighed amounts of food in excess of the daily requirement were provided. Scattered food on the filter-paper was separated from the faeces each morning, added to the food remaining in the feeding trough and weighed to 0·05 g. Since evaporation of water from the food was not measured, the figures for food intake are only approximate.

Water balance. The response to water administration (water diuresis) was tested as described by Schnieden & Blackmore (1955), i.e. the animals were given 5% of their body-weight of water by stomach tube and 1 or 2 h later another dose equivalent to 2·5 or 5% of their body-weight. Urine volumes were noted at 30 min intervals. The diuretic response was expressed as the percentage water load excreted in the 3 h after the second dose, water load being defined as the total volume of water given less the amount excreted during the hour after the first dose. In experiments in which substances were given parenterally the controls were injected with the appropriate volume of 0·9% NaCl solution or, in the experiments with cortisone and prednisolone, with the fluids in which these steroids were suspended. Total body water was estimated by cutting the carcass of the animal into small pieces (the gastro-intestinal tract was emptied) which were then dried to constant weight at 100°. Wintrobe's tubes and technique (Dacie, 1951) were used for haematocrit determinations.

Measurement of liver fat. Liver fat (total lipids) was estimated by a modification of the method of Bloor (1928) which was similar to that used by Best, Hartroft, Lucas & Ridout (1955); the liver is crushed in a glass mortar with 99% ethanol (3 ml./g tissue), transferred to a Soxhlet extractor with 150 ml. ethanol and refluxed overnight. The ethanol is then distilled off and the residue extracted three times with 20 ml. of a

mixture of light petroleum and chloroform (3:1, v/v) for 5 min at about 45°. The combined extracts are transferred to a tared beaker, evaporated to dryness and weighed. The same procedure was used to estimate total lipids in kidneys. A biuret method (Sinclair, 1947) was used to determine total plasma proteins and albumin. The results of electrophoretic estimations of the plasma-protein fractions will be discussed in a separate report. Plasma sodium and potassium were measured with a Lange flame photometer. Sulphosalicylic acid was used to test for protein in urine (King & Wootton, 1956).

Histological methods. Paraffin sections of tissues fixed in a solution of 4% (v/v) formaldehyde in 0·9% NaCl or in Helly's solution were stained with haematoxylin and eosin and Van Gieson's stain. Frozen sections of formalin-fixed liver and kidney were stained for fat with oil red O and counterstained with haematoxylin.

The amount of fat seen in the frozen sections of liver was assessed in arbitrary terms of + to + + + + in the same way as was done by Best *et al.* (1955).

All the tissues were examined and histological reports made by one of us (T.F.H.) objectively, in the first instance without knowing whether they were from controls or from the experimental animals, thus obviating any conscious bias.

Materials. The following preparations were used: aminophylline (Burroughs, Wellcome & Co.), acetazolamide (Diamox, Lederle Laboratories), Mictine (Searle & Co. Ltd), mersalyl (Salygran, Bayer Products Ltd), cortisone acetate (Cortisyl, Roussel Laboratories Ltd), and prednisolone (Delta-cortril, Pfizer Ltd).

RESULTS

Effects of the protein-deficient plantain diet on weanling rats

Food and nutrient intake. Table 1 shows that in terms of bulk the amount of food eaten by the animals on the plantain diet was two to three times greater than that of their litter-mate controls. This finding is in keeping with the experience (Bruce & Kennedy, 1951) that control of food intake in the normal rat depends on the calorie value and that dilution of a diet with inert constituents causes a compensatory increase in the bulk eaten. However, it will be seen that, in spite of the increased amounts eaten, the calorie intake (per animal) of the rats on the plantain diet remained lower than that of the controls, though the ratio was much more favourable than that between the daily protein ingestion of the two groups. In other words, in relation to the amounts eaten the plantain diet was deficient in calories and in protein but considerably more so in the latter. It was also deficient in choline and probably in other lipotropic substances (Table 1). Table 2 shows that it not only failed to sustain growth, but that it actually caused loss of body-weight. The arrest of growth was not due to lack of potassium of which both diets contained ample amounts (Table 1).

Changes in blood composition. The plantain diet produced a marked decrease in the concentration of plasma protein (Table 2). The decrease in albumin was more pronounced than that in globulin and probably for this reason total body water was increased, i.e. the rats became oedematous and water diuresis was impaired. The haematocrit values in the protein-deficient rats were significantly lower than those in

the controls (Table 2), which is likely to have been due to haemodilution rather than to anaemia (Aschkenasy, 1957), but circulating blood volumes were not estimated. Plasma-electrolyte concentrations were not markedly altered (Table 2) so that osmoregulation was apparently well maintained.

Table 1. *Mean values for daily food and nutrient intake of a group of weanling rats on plantain diet (eight animals with a mean initial weight of 63·3 ± 2·13 g) and a group of litter-mates on control diet (four animals with a mean initial weight of 59·3 ± 3·23 g). All animals were kept at 26·7° and 75 % relative humidity. Total period on diets, 27 days*

	Rats on plantain diet				Rats on control diet			
	First week		Last week		First week		Last week	
Food or nutrient	Intake/ 100 g body-weight	Intake/ rat	Intake/ 100 g body-weight	Intake/ rat	Intake/ 100 g body-weight	Intake/ rat	Intake/ 100 g body-weight	Intake/ rat
Diet (g)	52·5	29·5	54·8	24·6	14·3	11·8	10·9	13·1
Calories (Cal.)	41·8	23·5	43·5	19·5	42·2	34·8	32·0	38·4
Protein (g)	0·57	0·32	0·60	0·27	3·22	2·66	2·44	2·94
Potassium (m-equiv.)	46·6	26·2	48·6	21·8	65·7	54·2	41·7	50·1
Choline (mg)	0·21	0·12	0·22	0·10	—	—	—	—
Methionine (mg)	6·2	3·5	6·7	3·0	—	—	—	—

Table 2. *Mean values with their standard errors for effects of the plantain diet on weanling rats kept at 26·7° and 75 % relative humidity. The animals had been fed on the diet for 3–4 weeks. Figures in parentheses denote the number of rats*

	Rats on plantain diet	Litter-mates on control diet	Significance of difference between control and treated animals
Percentage change in body-weight from initial weight	−27·6 ± 1·0 (76)	+93·8 ± 5·3 (49)	—
Total plasma proteins (g/100 ml.)	5·4 ± 0·37 (7)	7·9 ± 0·25 (4)	< 0·001
Plasma albumin (g/100 ml.)	2·57 ± 0·149 (36)	4·26 ± 0·213 (24)	< 0·001
Total body water (g/100 g)	72·3 ± 0·28 (38)	68·5 ± 0·32 (22)	< 0·001
Water diuresis (water load excreted in 3 h) (%)	74·8 ± 2·35 (114)	102·1 ± 1·78 (89)	< 0·001
Haematocrit (percentage of R.B.C.)	28·0 ± 0·85 (18)	44·0 ± 0·51 (11)	< 0·001
Plasma sodium (m-equiv./l.)	164·4 ± 3·27 (23)	156·8 ± 3·63 (23)	> 0·1
Plasma potassium (m-equiv./l.)	4·50 ± 2·10 (13)	4·49 ± 0·28 (10)	> 0·9
Liver fat (total lipids) (g/100 g fresh weight)	17·9 ± 1·62 (33)	6·1 ± 0·14 (26)	< 0·001

No protein (i.e. < 0·25 mg/100 g/h) could be found in the urine of twenty rats tested before and after 3 weeks on the plantain diet. The urine samples for this purpose were collected for 4 h during the day; 24 h collections were avoided for fear of contamination. The possibility of nocturnal proteinuria can therefore not be excluded.

Necropsy findings. In none of the animals was there any free fluid in the serous cavities or any gross oedema. The livers of the rats that had been kept on the plantain diet mostly had a pale, fatty appearance but there were no subserous haemorrhages

and no scarring. The kidneys in all but one group showed no gross abnormality. In the exceptional group ten out of nineteen animals showed varying degrees of cortical haemorrhage and swelling.

Histological examination. The livers of seventy-three experimental animals and fifty-nine controls were examined. In the controls there were only four livers in which any free fat was found. In two of these it was quite negligible, in the third there was enough to suggest a centrolobular distribution, and in the fourth there was much centrolobular fat. In the experimental animals the amount of visible fat was considerable but varied (Table 3). In the livers classified from ± to + + + + in the table the distribution of the fat in twenty-seven was clearly centrolobular (Pl. 1, 1), in those with a widespread distribution there were usually a few cells round the periphery of the lobule which remained free from fat (Pl. 1, 2). In only two was there a portal preponderance. No fat cysts were observed although the droplets were often very large. There was no suggestion of cellular infiltration or fibrosis, but in some of the worst affected livers the cells in the periphery of the lobules showed regenerative activity; these regenerating cells did not contain fat.

Table 3. *Degree and lobular distribution of fatty changes in the livers of rats on plantain diet for 3–4 weeks*

Total no. of rats	No. of rats with fatty change of severity						No. of rats with lobular distribution of stainable fat		
	0	±	+	+ +	+ + +	+ + + +	Central	Portal	All zones
73	1	5	18	15	28	6	27	2	43*

* Two of these had slight portal preponderance, four had less fat in the mid-zone than in the portal or central, and many had a suggestion of less fat in the outer portal zone.

The kidneys, with the exception of those of one group, showed no trace of stainable fat in the tubules and differed in no respect from those of the controls. The exception was in a group of nineteen rats on plantain diet (see above) among which ten had the well-developed haemorrhagic and necrotic lesions of the kidneys found in young rats with choline deficiency; in some of these there were some droplets of stainable fat in the tubular epithelium. With this exception there was no fat in the renal tubular epithelium even in those animals with the most advanced fatty infiltration of the liver.

The pancreas was examined in six rats with fatty livers and showed no microscopic change.

Chemical estimations of total lipids in livers and kidneys. The fat content of the livers of the protein-deficient animals varied widely (range, 6·1–33·6% of wet weight; coefficient of variation, 53·0) which agreed with the histological findings. Since—to our knowledge—the treatment of all experimental animals was the same, these differences may be ascribed to individual variabilities in food intake. It is known (Best & Huntsman, 1934–5; Heller & Blackmore, 1953) for instance that a fall in food intake will cause a reduction in liver fat when fatty changes have been established by dietary means.

Total lipids were also estimated in the kidneys of six protein-deficient rats and six

litter-mate controls. No significant difference was found. However, the absolute amounts of fat in the kidneys of weanling rats were so small that the histological results must be regarded as more reliable.

Effects of diuretics and corticoids on the urine output of weanling rats on control diet and at ambient temperatures and humidities

The experiments summarized in Table 4 were aimed at finding doses of various diuretic agents which would produce a significant increase in urine output in healthy weanling rats with a standard water load. Aminophylline, acetazolamide and Mictine were found to be effective and reliable diuretics in such animals. No diuretic effect could be obtained with mersalyl, nor did mersalyl enhance the diuretic action of aminophylline or ammonium chloride. This experience with mersalyl in rats is not unusual. The diuretic dose range of organic mercurials in this species seems to be very narrow and their action quite inconstant (Lipschitz, Hadidian & Kerpcsar, 1933; Dicker, 1946; Cohen, 1953; Blackmore & Schnieden, 1957). Mersalyl could therefore not be used in the trials on rats on plantain diet.

It was hoped to establish dose-response curves from these results on healthy animals from which the relative potency of the diuretic agents could be determined according to the procedure of Greiner & Gold (1953). However, under the experimental conditions and in the dose range chosen none of the diuretics tested gave a linear log dose-effect relationship.

Cortisone and prednisolone had a well-marked diuretic action. The dose of prednisolone needed to produce a significant increase of urine output was about half of the minimum effective dose of cortisone.

Effects of diuretics and corticoids on the water diuresis of rats on plantain diet

The experiments to be described differ from those discussed in the previous section in two important respects. All animals were kept at 26·7° and 70–75% relative humidity for at least 3 weeks before a first diuresis test was performed to show the degree to which—as compared with litter-mates on control diet—water diuresis was impaired in the protein-deficient rats. All animals were then given a course of treatment with the various diuretic agents for 3 days and a second diuresis test was done. Hence, the response to water administration could be compared before and after treatment.

Table 5 shows that aminophylline, acetazolamide and Mictine had a significant diuretic effect on the oedematous rats on plantain diet. So had cortisone acetate in doses of 1·25 and 2·5 mg/100 g, and prednisolone in the dose of 0·6 mg/100 g. Table 5 shows also that in these experiments the various diuretic agents used had on the whole less pronounced effects on the non-oedematous control animals than on the rats on plantain diet. Though, for example (Fig. 1), the treatment with 2·5 mg cortisone restored the impaired water diuresis of the protein-deficient rats completely ($P > 0{\cdot}8$), it did not raise the urine output of the controls significantly.

Table 4. *Effects of diuretic drugs and steroids on urine output of weanling rats kept on the control diet at ambient temperatures and humidities*

Substance administered	Route and manner of administration	Dose (mg/100 g body-weight)	No. of animals*	Percentage difference between urine volume of treated and control animals (mean value with its standard error)	Significance of difference between control and treated animals *P*
Aminophylline	i.m. (1 h before second dose of water)	2	12 (12)	+18±4·9	<0·001
		3	30 (30)	+22±4·8	<0·001
		4	12 (12)	+25±7·4	<0·001
		6	6 (6)	+22±9·6	<0·01
Acetazolamide (Diamox)	p.o. (2 h before second dose of water)	5	6 (6)	+38±4·8	<0·001
		15	6 (6)	+31±5·4	<0·001
		25	6 (6)	+85±16·0	<0·001
		75	6 (6)	+50±11·2	<0·001
		125	23 (21)	+40±6·6	<0·001
Mictine	p.o. (1 h before second dose of water)	5	18 (17)	+20±3·9	<0·001
		7·5	12 (12)	+27±5·0	<0·001
		10	16 (17)	+31±5·2	<0·001
		15	18 (18)	+66±9·6	<0·001
		20	6 (6)	+24±11·2	<0·01
Ammonium chloride	p.o. (four doses for 2 days)	30	11 (12)	+23±4·7	<0·001
		40	6 (6)	+23±1·4	<0·001
		60	16 (18)	+46±3·9	<0·001
Mersalyl	i.m. (5 h before second dose of water)	0·0027†	30 (30)	+ 4±2·5	>0·3
Mersalyl plus ammonium chloride	i.m. (5 h before second dose of water)	0·0027†	18 (18)‡	− 2±2·6	>0·6
Mersalyl plus aminophylline	i.m. (5 h before second dose of water)	0·0027†	6 (6)§	− 4±5·4	>0·7
Cortisone acetate	i.m. (2 h before second dose of water)	1·25	5 (6)	+17±10·6	>0·1
		2·5	6 (6)	+32±10·0	<0·02
Prednisolone	i.m. (2 h before second dose of water)	0·3	12 (12)	− 9±7·4	>0·4
		0·6	24 (23)	+16±6·6	>0·1
		1·2	12 (12)	+58±15·8	<0·001

i.m., intramuscularly; p.o., *per os*.
* Figures in parentheses are the number of control animals.
† m-moles/100 g.
‡ The control as well as the 'mersalyl' rats received four doses of 40 mg ammonium chloride/100 g for 2 days.
§ The control as well as the 'mersalyl' rats received 3 mg/100 g aminophylline 1 h before the second dose of water.

Table 5. *Effects of drugs and corticoids on water diuresis. The rats on the control diet were litter-mates of the rats on plantain diet. All animals were kept at 26·7° and 75% relative humidity*

Treatment	Dose (mg/100 g)	No. of animals	Difference between percentage water load excreted before and after treatment (mean value with its standard error)	Significance of difference *P*
		Rats on plantain diet		
Aminophylline	3	10	+31±9·8	<0·05
Acetazolamide	15	6	+56±19·1	<0·05
Mictine	15	6	+51±21·1	<0·05
Cortisone acetate	1·25	9	+44±17·8	<0·05
Cortisone acetate	2·5	9	+48±7·6	<0·01
Prednisolone	0·6	14	+21±4·1	<0·01
Prednisolone	1·2	6	+9±21·1	>0·4
		Rats on control diet		
Aminophylline	3	7	+9±3·7	>0·4
Acetazolamide	15	5	+30±14·8	<0·05
Mictine	15	5	+27±21·7	>0·1
Cortisone acetate	1·25	5	−5±9·6	>0·3
Cortisone acetate	2·5	9	+8±3·7	>0·2
Prednisolone	0·6	6	+25±11·5	<0·02
Prednisolone	1·2	6	+18±6·4	>0·1

A 'water-diuresis test' was done before the treatment was begun. The animals then received the first dose of the drug or steroid and four further doses on the next 2 days. A last dose was given before the water diuresis test was repeated. The routes of administration were as given in Table 4.

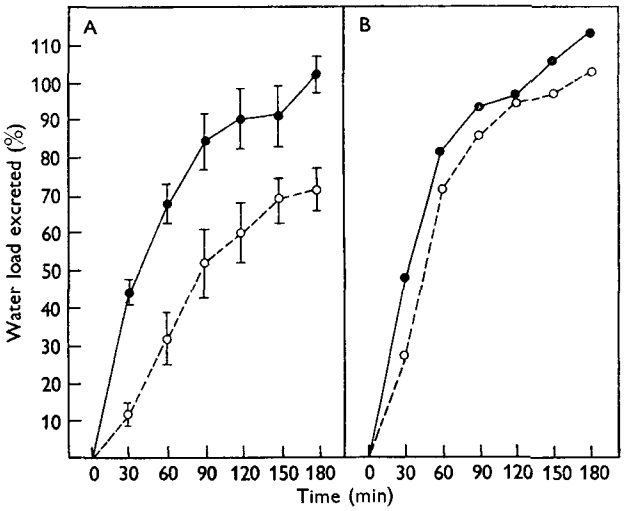

Fig. 1. Effect of cortisone on water diuresis. A, weanling rats on plantain diet for 4 weeks; B, litter-mate controls. o- - -o, before cortisone; ●——●, after cortisone (2·5 mg cortisone acetate/100 g body-weight twice daily for 3 days).

Effects of diuretics and corticoids on total body water

Table 6 shows that the three diuretics used did not lower the body water of either the protein-deficient rats or their controls significantly, whereas cortisone had a pronounced effect in both groups. In fact, treatment with 1·25 or 2·5 mg cortisone/100 g twice daily for 3 days reduced the body water of the 'plantain rats' to values which were much the same as those of untreated controls with normal plasma protein levels ($P > 0·1$ and $> 0·2$). Treatment with cortisone raised the food intake of the rats on plantain diet. For example, in a group of rats injected with 2·5 mg/100 g twice a day, the mean weight of food (g/100 g/24 h) was 75 g on the 1st day of treatment, whereas

Table 6. *Effects of diuretics and cortisone on total body water*

(All treated animals received two doses of the drug or hormone for 3 days and a last dose 8–12 h before they were killed. The controls were litter-mates of the rats on plantain diet. Mictine and acetazolamide were given by mouth, aminophylline and cortisone acetate by intramuscular injection. All animals were kept at 26·7° and 75 % relative humidity)

Treatment	Dose (mg/100 g)	No. of animals	Body water (g/100 g body-weight) (mean value with its standard error)	Significance of difference between treated and untreated groups P
		Rats on plantain diet		
Untreated	—	26	72·1 ± 0·31	—
Aminophylline	3	8	73·2 ± 0·43	> 0·1
Acetazolamide	15	6	71·2 ± 0·98	> 0·2
Mictine	15	6	71·7 ± 0·81	> 0·5
Cortisone	1·25	12	70·3 ± 0·55	< 0·01
Cortisone	2·5	12	70·1 ± 0·48	< 0·01
		Rats on control diet		
Untreated	—	10	69·2 ± 0·41	—
Aminophylline	3	6	70·0 ± 0·46	> 0·3
Acetazolamide	15	4	68·4 ± 0·68	> 0·3
Mictine	15	4	68·3 ± 0·29	> 0·2
Cortisone	1·25	7	67·5 ± 0·33	< 0·01
Cortisone	2·5	8	67·5 ± 0·33	< 0·01

Table 7. *Effects of prednisolone on total body water*

(All treated animals received two doses of the steroid intramuscularly for 3 days and a last dose 8–12 h before they were killed. The controls were litter-mates of the rats on plantain diet. All animals were kept at 26·7° and 75 % relative humidity)

Treatment	Dose (mg/100 g)	No. of animals	Body water (g/100 g body-weight) (mean value with its standard error)	Significance of difference between treated and untreated groups P
		Rats on plantain diet		
Untreated	—	12	72·6 ± 0·57	—
Prednisolone	0·6	14	69·8 ± 0·50	< 0·01
Prednisolone	1·2	7	71·6 ± 0·76	> 0·2
		Rats on control diet		
Untreated	—	18	68·3 ± 0·34	—
Prednisolone	0·3	6	68·4 ± 0·46	> 0·9
Prednisolone	0·6	18	67·8 ± 0·34	> 0·3
Prednisolone	1·2	12	67·8 ± 0·93	> 0·5

untreated controls ate only 57 g. This effect of cortisone decreased on the 2nd and 3rd days (2nd day, 68 g as against 60 g; 3rd day, 60 g as against 57 g).

Results with prednisolone are shown in a separate table (Table 7) because the values for total body water in the controls used in these experiments were somewhat lower than those of the control animals in the experiments recorded in Table 6. The work with prednisolone was done some 6 months later than that with cortisone and the difference between the controls may have been due to seasonal influences. However, the difference between untreated protein-deficient rats and controls remained highly significant ($P < 0{\cdot}001$). It will be seen that the larger dosage of prednisolone failed to lower significantly total body water of the protein-deficient rats (it will be noted in Table 5 that it had also failed to produce a water diuresis in such animals), but the smaller dose was effective. In contrast to cortisone, prednisolone did not decrease the body water of the controls.

DISCUSSION

There is by now sufficient evidence to suggest that impairment of water diuresis will occur whenever a diet is severely deficient in protein. It has also been observed in adult rats on low-casein diets (Dicker, Heller & Hewer, 1946) or fed on carrots (Guggenheim & Hegsted, 1953) or cassava (Schnieden, Hendrickse & Haigh, 1957). Estimation of total body water seems a simple and valid procedure to distinguish between the occurrence of oedema and mere shifts in body water which may occur in undernutrition in adults (Widdowson & McCance, 1951) and in marasmic infants (Kerpel-Fronius & Frank, 1949). Our figures for total body water in control animals are somewhat higher than those reported by Da Costa & Clayton (1950) who like us used a desiccation method (though in older animals) and approach those found for the deuterium space in adult rats (Haigh & Schnieden, 1956). It cannot be said from our work to what degree the changes in the water metabolism of rats on the protein-deficient plantain diet should be ascribed to alterations in kidney function; proteinuria could not be demonstrated and no fatty changes in the renal tubules were seen. The latter results are of some interest in view of the report of the constant occurrence of fatty changes in the cells of the convoluted tubules in infants with kwashiorkor in Uganda (Davies, 1956). In one group of protein-deficient rats only, haemorrhagic kidney lesions were found which closely resembled those seen in choline deficiency. The plantain diet used was not only deficient in protein but also in lipotropic substances (Table 1). It may be assumed that ordinarily it contained just enough choline to prevent haemorrhagic kidneys (Griffith & Wade, 1939), but that in the exceptional group requirements for lipotropic substances (which are influenced by other dietary constituents) (Beveridge, Lucas & O'Grady, 1945) happened to be higher or that the batch of plantains used contained less of the lipotropic factors.

Best *et al.* (1955) have found that in rats choline deficiency causes accumulation of lipids in the centre of the liver lobule, whereas protein deficiency or amino-acid imbalance produces fatty infiltration which first appears in the periportal areas. In their experiments a diet low in vegetable protein (soya protein 2% and peanut protein 1%), which contained only 32 mg methionine/100 g, unsupplemented with choline

produced a very fatty liver, some of the lipid appearing in periportal areas but most of the abnormal fat being centrolobular. Our results with rats fed on the plantain diet which contained even less protein and methionine, and only traces of choline, show a very similar distribution of hepatic fat. These findings differ from those of Shils, Friedland & Stewart (1954) who reported a periportal accumulation of lipids in rats fed on maize, rice, wheat or cassava, and who believed that this distribution is typical with diets low in vegetable protein. However, the present results together with those of Best *et al.* (1955) suggest that the effect of a protein-poor diet on the distribution of liver fat will be influenced not only by the amount and composition of protein which it contains, but also by its content of lipotropic substances. Since no microscopic changes were found in the pancreas in six of the rats with very fatty livers we did not examine the remainder. It is likely that the absence of pancreatic atrophy and of fibrotic change in the livers—two features regularly observed in kwashiorkor in Uganda—was due to the relatively short time (3–4 weeks) for which the animals had been kept on the protein-deficient diet.

Transient diuretic effects could be produced in the protein-deficient rats with aminophylline, acetazolamide and Mictine, but treatment with these drugs for 3 days did not lower significantly the increased body water of such animals.

It is well known (Winter, 1952; Dexter & Stoner, 1952) that cortisone enhances the urine output of normal rats. This was also found in the present investigation. The diuretic effect in animals kept on control diet, but at ambient temperatures and humidities was more pronounced than in control rats kept in an artificial tropical climate. However, treatment with cortisone for 3 days sufficed to lower significantly the total body water in the latter group. This observation may partly explain Winter's (1952) findings that treatment with cortisone increased the daily urine volume without an increase of water intake.

Treatment with cortisone of the rats with nutritional oedema was more effective than in the controls (in keeping perhaps with the clinical experience that diuretics act better in oedematous patients). The impaired water diuresis of the protein-deficient animals was completely restored. Their body water dropped to values which were not significantly different from those of the controls. In other words, treatment with cortisone freed the animals of oedema.

Prednisone and prednisolone have been reported (Pequet, 1955; Nabarro, Stewart & Walker, 1955) to cause less retention of sodium than cortisone and to be effective in the treatment of nephrotic oedema (McCrory & Macaulay, 1957). The two steroids are equally potent in increasing the water diuresis of adrenalectomized rats (Chart, Hetzel & Gaunt, 1956), but prednisolone has been found to be more active in antagonizing the antidiuretic effect of Pitressin in intact animals (Gaunt, Lloyd & Chart, 1957). In our experiments, prednisolone increased renal water excretion in normal animals kept at ambient or at 'tropical' temperatures. It also increased the urine volume of the oedematous rats. But although lower doses of prednisolone than of cortisone seemed to be effective, the diuretic effects obtained with prednisolone were smaller than those seen with cortisone. The action of prednisolone on total body water was, on the whole, in agreement with these results. Treatment for 3 days did not lower

the body water of the controls significantly, but 0·6 mg/100 g given twice daily to the protein-deficient rats had a significant effect. It would seem, therefore, that in oedema of protein deficiency prednisolone cannot be ruled out as a diuretic agent for clinical trial.

It is as difficult to account for the efficacy of the steroids (as contrasted with 'diuretics') in experimental nutritional oedema as in the somewhat similar condition of oedema of nephrosis. Adult rats kept on a diet with similarly low protein content have been shown to have a low glomerular filtration rate (Dicker, 1950; Schnieden & Blackmore, 1957). Cortisone raises the filtration rate of such animals, which may have been the cause of its diuretic action. However, theophylline, which affects renal function in a like manner, did not lower the body water of our oedematous animals. It could be argued that this was due to a more intermittent effect of aminophylline as compared with the more protracted action of cortisone. Another possibility is that the metabolic effects of cortisone come into play; treatment with cortisone raised the food intake of the protein-deficient animals and has also been shown (Silber & Porter, 1953) to mobilize protein from the tissues. Both effects may have caused a rise in plasma proteins which would have tended to increase water excretion.

SUMMARY

1. Two hundred and thirty-two weanling rats were kept at tropical temperature and humidity and fed for 3–4 weeks on a diet of boiled African plantains which was deficient in protein and lipotropic substances but provided sufficient potassium.

2. The following changes were observed in such animals when compared with litter-mates kept on a fully balanced control diet: growth was arrested, the concentration of plasma albumin was decreased, haematocrit values were low, total body water was increased and water diuresis was impaired. There were fatty changes in the liver, the distribution being in most instances centrolobular but there was no fibrosis. There was no evidence, in the animals examined, of any atrophic changes in the pancreas, and no animal had diarrhoea. With the exception of one small series of rats on the plantain diet there were no microscopic changes of any kind in the kidneys and no proteinuria was observed. The exceptions were ten rats with the gross haemorrhagic renal lesions of choline deficiency; it was only in these that even a trace of stainable fat was detected in the tubular epithelium. The plasma sodium and potassium levels were not significantly altered.

3. Aminophylline, acetazolamide (Diamox), Mictine, cortisone and prednisolone increased water diuresis in the protein-deficient animals, but only cortisone raised it to the level of healthy controls.

4. When the protein-deficient animals were treated with the diuretics and the steroids for 3 days, only the steroids produced a marked decrease in total body water. Cortisone reduced the body water of the oedematous rats to levels which were not significantly different from those of untreated animals on the control diet.

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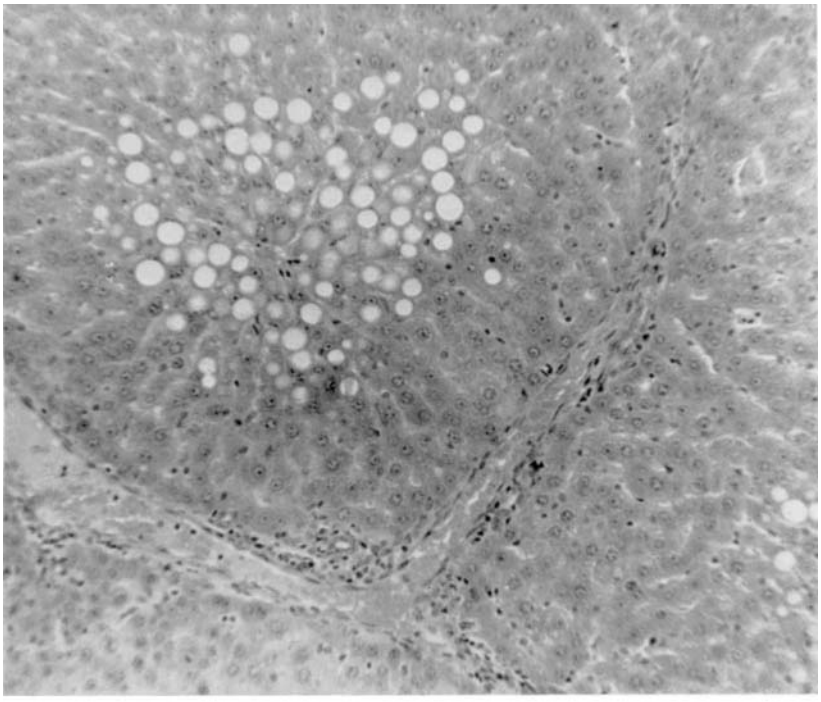
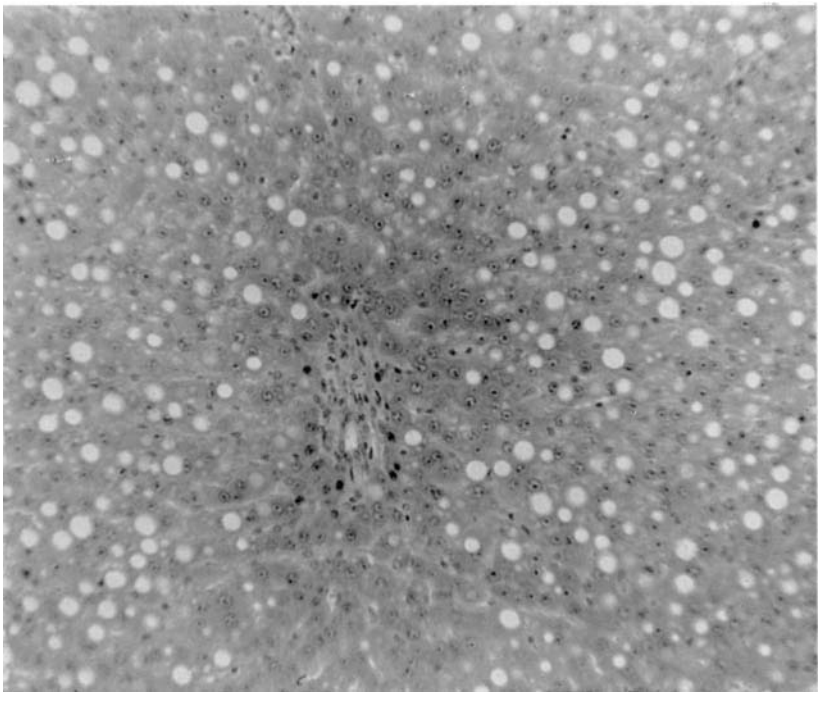
EXPLANATION OF PLATE

1. Liver of rat on plantain diet for 3 weeks. Centrolobular fatty infiltration (+). × 120.
2. Liver of rat on plantain diet for 3 weeks. A slightly greater degree (+ +) of fatty infiltration than that shown in 1 but still with less fat around the portal area, in the centre of the photograph, than towards the centre of the lobules. The liver cells in this portal area show nuclear activity. × 120.

Plate 1



1



2

(*Facing p.* 64)