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THE EFFECT OF DIURETICS ON THE WATER EXCRETION OF PROTEIN DEFICIENT RATS

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Adult rats kept for eleven weeks on a diet deficient in protein lost weight and some developed scrotal oedema. The retention of bromsulphthalein was increased, but the thymol turbidity test was unaffected; the apparent plasma volume was increased.

Water diuresis in the protein deficient animals was impaired. There was no apparent delay in the mean rate of water absorption from the whole gastro-intestinal tract although a delayed absorption of water from the intestine was found in some animals. The concentrations of total plasma proteins and plasma albumin were low as compared with normal animals, but the plasma sodium levels were within normal limits. The inulin clearance (glomerular filtration rate) of the animals on the protein-deficient diet was significantly lower than that of the controls.

In normal rats, aminophylline and acetazolamide were diuretic. Caffeine and sodium benzoate did not increase the urine output and mersalyl was antidiuretic. In the protein deficient rats, cortisone acetate increased the water diuresis. Caffeine and sodium benzoate, aminophylline and acetazolamide did not significantly increase this response, mersalyl had an antidiuretic effect. Cortisone acetate increased the food and water intake of the protein deficient rats; it also increased the glomerular filtration rate.

It is now well established that animals kept on low protein diets have an impaired water diuresis (Dicker, Heller, and Hewer, 1946; Heller and Dicker, 1947; Leslie and Ralli, 1947; Guggenheim and Hegsted, 1953; Schnieden and Blackmore, 1955a). Rats kept on a protein deficient diet also have an increased total body water and are oedematous (Haigh and Schnieden, 1956). We have investigated the effects of diuretics on such animals to see whether water diuresis could be restored. In addition to the conventional drugs (mercurial and xanthine diuretics), cortisone and corticotrophin were also tested because these hormones have a diuretic effect in nephrosis (Luetscher, Deming, Harvey, Lew, and Poo, 1950; Luetscher, Deming and Johnston, 1951). Nephrosis, like nutritional protein deficiency, is characterized by a low plasma protein level and oedema.

METHODS

Animals.—Male albino rats weighing 180 to 240 g. were used. Diets and water were unrestricted.

Control Diet.—This consisted of: wheat seed 22.7, barley meal 19.5, linseed cake (foreign) 19.5, decorticated ground nut meal 19.5, dried separated milk 2.4, white fish meal 4.8, meadow hay 9.8, cod-liver oil 1.8. This diet contained 25.6% protein (crude), 3.9% fat (ether extract), and 45.2% carbohydrate (Woodman, 1952). It provided approximately 330 cal./100 g. and 7.8 g. protein (crude)/100 cal.

Protein Deficient Diet.—This consisted of: casein 0.5, maize starch 80, hardened ground nut oil 12, Steenbock's salt mixture (Hawk and Bergeim, 1942) 4.5, cod liver oil 3.0, supplemented by 50 mg. vitamin B complex powder (Crookes Laboratories/100 g. diet. It contained 0.4% thiamine, 0.5% riboflavine, 0.18% pyridoxine, 4% nicotinamide, and 0.0014% pantothenic acid. 3 mg. tocopherol acetate/100 g. diet was also added. This diet provided approximately 300 cal./100 g. and 0.2 protein/100 cal.

Measurement of Food Intake.—The daily intake of food was estimated by placing groups of 6 rats in suspended cages. Weighed amounts of food in excess of the daily requirement were provided. Some scattering of food could not be prevented, so the cages were suspended over a tray lined with filter paper and the scattered food separated from the faeces each morning and added to food not consumed.

Measurement of Water Intake.—Rats were placed in individual metabolism cages fitted with graduated drinking tubes. The amount of water drunk by each animal in 24 hr. was noted.

Plasma Proteins.—A biuret method was employed for estimation of total plasma protein and plasma albumin (Hiller, 1927; Peters and Van Slyke, 1932).

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Plasma Sodium.—Heparinized plasma was obtained from blood taken from a carotid artery. A flame photometer was used for the estimation of sodium.

Plasma Volume (Evans Blue Space).—This was estimated according to the procedure used by Ginsburg (1954). The rats were allowed food and water up to the beginning of the experiment. They were then anaesthetized with ether and cannulae were inserted into a jugular vein and a carotid artery. Evans blue was injected into the jugular vein and blood samples were obtained from the carotid artery.

Haematocrit Estimations.—Wintrobe's technique and tubes were used.

Liver Function Tests.—Thymol turbidity tests were done according to McLagan (1944). Bromsulphthalein retention in rats was estimated by a modification of the method of Ahmed and Frazer (1952). A polythene cannula was inserted into the external jugular vein of the rat under ether anaesthesia and brought out through the skin between the ears (see Ginsburg and Heller, 1953). The animal was later allowed access to food and water until the start of the test 24 hr. later. Bromsulphthalein (25 mg./kg. body weight) was injected intravenously and 25 min. later the animal was quickly anaesthetized with ether. A siliconed polythene cannula was inserted into the carotid artery. Blood was collected from the carotid artery into heparinized tubes exactly 30 min. after the bromsulphthalein injection. The technique used makes it possible to obtain plasma completely free from haemolysed cells. The dye concentration in the plasma was estimated electrophotometrically.

Response to Water Administration (Water Diuresis).—Rats were randomly divided into groups before being put on diet and kept under identical environmental conditions before and during the experiments. The procedure of Schnieden and Blackmore (1956) was used.

Gastro-intestinal Absorption.—The procedure described by Schnieden and Blackmore (1955b), which is based on the method of Heller and Smirk (1932), was used. The animals were killed 45 min. after administration of their second dose of water.

Insulin Clearance (Glomerular Filtration Rate).—The method of Dicker and Heller (1945) was used, but more water was given so that the estimation was carried out when the animal had a water load of approximately 7% of its body weight. Inulin was estimated by the resorcinol method of Schreiner (1950).

Materials.—Corticotrophin (Crookes), cortisone acetate (Merck) and cortisone suspending fluid were supplied by the Medical Research Council. Other preparations used were mersalyl (Bayer), aminophylline (Burroughs Wellcome), caffeine and sodium benzoate (Ferris), acetazolamide (Lederle), inulin (Kerfoot), and heparin (Evans).

Results

Effects of Protein Deficient Diet

Rats which had been on the protein deficient diet for 11 weeks were apathetic and smaller than the controls. Some had scrotal oedema. Faeces were well-formed but of soft consistency. The mean body weight was about half that of the controls (Table I); most of this difference was due to a loss of weight of the dieted rats rather than to a gain in weight of the controls. The loss of weight in the first 5 weeks averaged 60.2 ± 4.6 g., and in the last 5 weeks 31.6 ± 5.1 g. The decreasing rate of weight loss may in part be due to the developing oedema (Haigh and Schnieden, 1956). Plasma protein and plasma albumin were reduced by the diet and the plasma volume was increased. There was a delayed bromsulphthalein excretion but the thymol turbidity test was normal. Plasma sodium was not changed. Water diuresis decreased as the diet was maintained, the mean time for 50% excretion of the water load increasing from 63 min. initially to 84, 116 and 171 min. after 5, 8, and 11 weeks on the diet. This was not due to a delay in water absorption from the gastro-intestinal tract, but there may have been a small delay in absorption from the gut itself (Table II). The main reason for failing water diuresis was a decrease in the glomerular

TABLE I

EFFECT ON ADULT RATS OF FEEDING A PROTEIN DEFICIENT DIET FOR ELEVEN WEEKS

The values given are for the mean ± S.E. Numerals in brackets are the numbers of animals in each group.

	High Protein Diet	Protein Deficient Diet
Body weight (g.)	210 ± 2 (24)	110 ± 9 (24)
Total plasma protein (g./100 ml.)	6·2 ± 0·6 (10)	4·3 ± 0·1 (21)
Plasma albumin (g./100 ml.)	4·3 ± 0·1 (8)	2·5 ± 0·2 (19)
Thymol turbidity test (units)	0–4 (12)	0–4 (12)
Bromsulphthalein test (% retention)	5·7 ± 0·5 (9)	39·7 ± 4·0 (10)
Haematocrit (% R.B.C.)	41·1 ± 4·0 (34)	28·5 ± 1·9 (20)
Evans blue space (ml./100 g.)	4·0 ± 0·1 (16)	6·5 ± 0·5 (10)
Blood volume (ml./100 g.)	6·8 ± 0·1 (16)	7·4 ± 0·2 (5)
Plasma sodium (m.eq./l.)	146 ± 1·4 (13)	145 ± 3·1 (12)

TABLE II

GASTRO-INTESTINAL WATER ABSORPTION IN PROTEIN DEFICIENT RATS AND CONTROLS

The values given are % of water retained (mean ± S.E.). Numerals in brackets give the numbers of animals/group.

% of Dose Retained in:	Controls	Protein Deficient Rats	*t*	*P*
Stomach	6·2 ± 1·4 (12)	6·3 ± 1·6 (10)	0·1	> 0·9
Intestine	8·2 ± 1·4 (12)	18·2 ± 5·2 (10)	2·0	< 0·05
Gastro-intestinal tract	14·3 ± 1·7 (12)	24·5 ± 6·3 (10)	1·6	> 0·1

filtration rate, which fell from 0.82 ± 0.8 (7) to 0.32 ± 0.06 (7) ml./100 g./min. Animals on the diet also reduced their fluid intake, which fell from 15.8 ± 0.9 (6) to 4.1 ± 0.8 (6) ml./100 g./24 hr.

Actions of Corticotrophin and Cortisone on Protein Deficient Rats

Corticotrophin had no effect at doses of 2.5 U./100 g. twice daily for 3 days given subcutaneously. Cortisone acetate given twice daily by subcutaneous injection for 3 days had the effects shown in Figs. 1 and 2. At doses of 2.5 mg./100 g. cortisone greatly improved diuresis. This change was accompanied by an improvement in appetite (Fouts, 1943; Meiklejohn and Passmore, 1951; Trowell, Davies, and Dean, 1952; Heller and Blackmore, 1953; Thompson, 1954), the food intake rising from 3.8 ± 0.3 (12) to 5.7 ± 0.5 (12). In spite of this increased food intake, the animals lost weight.

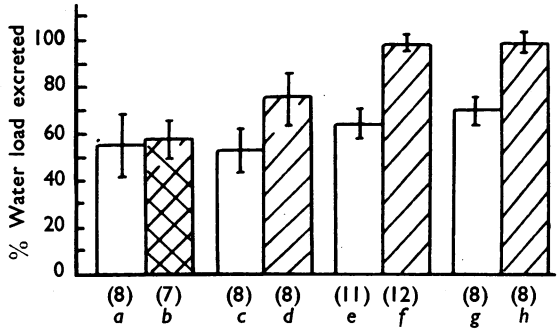

FIG. 1.—The effect of cortisone acetate on % water load excreted in 3 hr. (mean ± S.E.) by rats kept for 11 weeks on a protein deficient diet. (The numerals in brackets give the numbers of animals/group.) (*a*), (*c*), (*e*), and (*g*), control using cortisone suspending fluid. (*b*), control using 0.9% sodium chloride solution. (*d*), cortisone acetate, 1.25 mg./100 g. (*f*), cortisone acetate, 2.5 mg./100 g. (*h*), cortisone acetate, 3.75 mg./100 g.

The same dose of cortisone acetate increased the water intake of the protein deficient animals. The water intake of rats injected with cortisone suspending fluid for a period of 2 days was increased from 4.6 ± 0.5 to 7.1 ± 1.0 ml./100 g./24 hr.

There was an increase in inulin clearance but not in tubular reabsorption of water (Table III).

TABLE III

INULIN CLEARANCE AND TUBULAR REABSORPTION OF WATER IN PROTEIN DEFICIENT RATS TREATED WITH CORTISONE ACETATE OR WITH CORTISONE SUSPENDING MEDIUM

Values given are for mean ± S.E. Numerals in brackets are numbers of animals in each group. 2·5 mg./100 g. of cortisone acetate was given twice daily for 3 days. Tubular reabsorption of water = inulin clearance − urine flow in ml./100 g./min.

Treatment	Inulin Clearance		Tubular Reabsorption	
Injection with suspending medium	$0{\cdot}34 \pm 0{\cdot}07$ (14)	$t = 2{\cdot}3$, $P < 0{\cdot}02$	$80{\cdot}5 \pm 3{\cdot}7$ (6)	$t = 0{\cdot}8$, $P > 0{\cdot}4$
Injection with cortisone acetate	$0{\cdot}58 \pm 0{\cdot}08$ (12)		$84{\cdot}4 \pm 3{\cdot}2$ (6)	

Effect of Caffeine, Aminophylline, Acetazolamide, and Mersalyl on the Water Diuresis of Normal and Protein Deficient Rats

Normal Rats.—The results are shown in Table IV. Caffeine and sodium benzoate (10 mg./100 g.) were given twice daily intramuscularly for 3 days. No significant change in water diuresis resulted from this treatment. Acetazolamide was given once a day by stomach tube in a dose of 125 mg./100 g. At the end of the 180 min. period of observation % water load excreted by the rats treated with acetazolamide did not differ significantly from the controls, but there was a significant difference during the first 90 min. (% water load excreted by controls was 55.1 ± 4.3 (6)

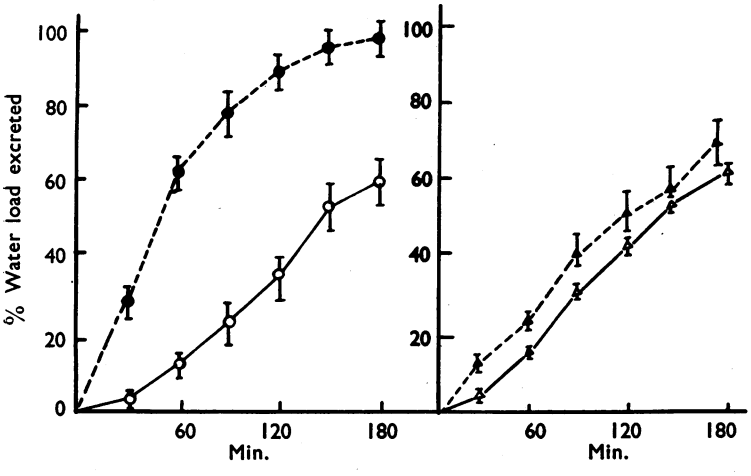

FIG. 2.—The effect of cortisone acetate (2.5 mg./100 g.) on the water diuresis of rats kept for 11 weeks on a protein deficient diet (mean ± S.E. of group of 12 rats). ○ Control before cortisone acetate. ● After cortisone acetate. △ Control before cortisone suspending fluid. ▲ Control with cortisone suspending fluid.

TABLE IV

EFFECT OF DIURETICS ON WATER EXCRETION OF NORMAL RATS

Numerals in brackets are numbers of animals in each group. Controls were injected with 0·9% saline. Treated rats were those receiving the diuretic.

Diuretic (for Doses see Text)	% Water Load Excreted in 3 Hr. by Normal Rats		t	P
	Treated Rats	Controls		
Caffeine and sodium benzoate	90·7±1·4 (6)	87·9±5·3 (6)	0·5	>0·5
Aminophylline ..	109·8±5·6 (6)	87·9±5·3 (6)	2·8	<0·01
Acetazolamide ..	93·4±1·6 (6)	87·9±5·3 (6)	1·0	>0·3
Mersalyl	63·1±3·7 (6)	95·0±5·7 (6)	3·4	<0·001

compared with 71.4±0.7 (6) for rats treated with acetazolamide: $t=3.7$; $P<0.001$). Aminophylline (4.0 mg./100 g. given intramuscularly twice daily for 3 days) had a significant diuretic effect. Mersalyl has been stated to exert its maximum diuretic effect in rats when given in a dose of 0.0027 ml./100 g. body weight 10 hr. before the diuresis test (Dicker, 1946). A single dose of mersalyl was therefore given intramuscularly following this procedure. There was no diuresis and in fact an antidiuretic effect was observed.

Protein Deficient Rats.—The same doses of all diuretics were used. Caffeine and sodium benzoate again failed to increase urine output. When a higher dose (16.7 mg./100 g. twice daily intramuscularly for 3 days) was used in a group of 7 animals, 3 died and the urine output of the remaining animals was similar to that of the controls. Neither acetazolamide nor aminophylline had any significant effect on water diuresis in the protein deficient rats. Mersalyl had again an antidiuretic effect (Table V).

TABLE V

EFFECT OF DIURETICS ON WATER EXCRETION OF RATS KEPT ELEVEN WEEKS ON A PROTEIN DEFICIENT DIET

Numerals in brackets are numbers of animals in each group. Treated rats received a diuretic. Control rats received (*a*), 0·9% sodium chloride solution; (*b*), cortisone suspending fluid; and (*c*), an injection contaminated with posterior pituitary gland hormones.

Diuretic (for Doses see Text)	% Water Load Excreted in 3 Hr. by Protein Deficient Rats		t	P
	Treated Rats	Controls		
Caffeine and sodium benzoate	54·6± 5·7 (10)	(*a*) 53·8±5·3 (9)	0·1	>0·9
Aminophylline ..	86·4± 6·8 (11)	(*a*) 74·4±9·3 (12)	1·0	>0·3
Acetazolamide ..	64·7±10·0 (12)	(*a*) 74·4±9·5 (12)	0·8	>0·4
Mersalyl	33·4±13·1 (12)	(*a*) 65·2±8·1 (10)	4·4	<0·001
Cortisone acetate ..	97·0± 7·0 (12)	(*b*) 65·4±6·4 (12)	3·6	<0·001
Corticotrophin ..	13·0± 2·9 (12)	(*c*) 50·6±9·4 (12)	4·3	<0·001

DISCUSSION

If we assume that the main reason for the impaired water diuresis of protein deficient rats is the observed decrease in glomerular filtration rate, an effect also noted by Dicker (1950) under similar conditions, diuretics like mersalyl and acetazolamide, acting exclusively or mainly on the tubules, would not be expected to produce a diuretic effect. However, xanthine diuretics such as aminophylline which has been reported to influence blood flow in the rat kidney (Dicker, 1946) also failed to increase water diuresis in our protein deficient animals. In contrast to this failure with the more conventional diuretics are the results with cortisone which raised the water diuresis of the protein deficient rats to levels well comparable to that of controls which had been kept on a high protein diet. Cortisone is known to increase water diuresis in normal rats (Winter, 1952; Dexter and Stoner, 1952). It has also been shown to increase the glomerular filtration rate and renal blood flow in healthy human beings (Ingbar, Relman, Burrows, Kass, Sisson and Burnett, 1950; Levitt and Bader, 1951) and the glomerular filtration rate in nephrosis (Deming and Luetscher, 1950; Luetscher *et al.*, 1950). In view of our negative results with the xanthine diuretics (especially since aminophylline in the dose used had a diuretic effect in normal rats) the glomerular effect of cortisone is probably not the only factor involved in its action. Silber and Porter (1953) have shown that cortisone causes a decrease in carcass proteins in adrenalectomized protein deficient rats but an increase in liver and plasma protein. This increase in plasma proteins may be an important factor in the diuretic action of cortisone demonstrated in our experience, because in nephrosis and in hepatic cirrhosis, in which, as in protein deficiency, the plasma albumin concentration is low, intravenously administered albumin has a diuretic effect and increases glomerular filtration rate (Luetscher, Hall, and Kremer, 1949; Orloff, Welt, and Stowe, 1949; Chinard, Lauson, Eder, Greif, and Hiller, 1954; Patek, Mankin, Colcher, Lowell, and Earle, 1948). It may well be that a combination of the metabolic effects of cortisone and its action on renal

blood flow and glomerular filtration rate are needed to influence the impaired water diuresis in protein deficient animals.

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