Effect of Protein Energy Malnutrition on the Renal Function and Extracellular Fluid Volume of the Rat

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SUMMARY

Chromium (^{51}Cr)-EDTA was used to estimate extracellular fluid volume and glomerular filtration rate of the rat. In rats with protein energy malnutrition and oedema (kwashiorkor), there was a significant increase in extracellular fluid volume (30 cf 24 ml/100 g), but no change in glomerular filtration rate. An infusion of angiotensin (0,3 to 1,4 ng/g/min) produced a decrease in both glomerular filtration rate and in the rate of urine formation, suggesting that an increase in the activity of the renin-angiotensin system may contribute to the development of oedema associated with protein energy malnutrition, by opposing the excretion of urine by the kidneys.

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A decrease in the glomerular filtration rate (GFR)[1-3] possibly associated with an increase in plasma renin activity, may be responsible for the fluid retention and expansion of the extracellular fluid (ECF) volume,[4] which are cardinal features of the protein energy malnutrition disease, kwashiorkor. The present investigation is to determine whether protein energy malnutrition can produce comparable changes in the rat, and whether these changes could result from an increase in plasma renin activity and the consequent formation of angiotensin.

METHODS

The volume of distribution of ^{51}Cr-EDTA was compared with that of thiosulphate in a preliminary experiment on 6 unanaesthetised, restrained rabbits weighing 3,2 - 5,3 kg. Three ml of a solution containing 300 mg sodium thiosulphate and 30 μCi chromium (^{51}Cr)-EDTA (Amersham CJ 13P) were injected over a 2-min period into a right ear vein, and blood samples were collected from the left ear veins at intervals between 2 and 80 min after injection. Heparinised plasma was separated and its thiosulphate concentration[5] and radio-activity (Packard Auto-Gamma spectrometer) were compared with a 1/200 dilution of the injected fluid.

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Forty-eight rats weighing from 115 to 219 g, and fed on a standard diet of rat cubes (Rhodesian Milling Company) containing 22% protein, were anaesthetised with pentobarbitone (40 μg/g, by intraperitoneal injection) and infused with mannitol solution (100 g/litre) through a jugular vein cannula at 150 μl/min. Thirty minutes later the rats were heparinised (1 unit/g by intravenous injection) and the infusion was interrupted for 10 sec while approximately 5 μCi of ^{51}Cr-EDTA in 0,1 ml of saline was rapidly injected (3 sec) from a weighed syringe. Arterial blood samples of about 50 μl were collected from a cannulated carotid artery at intervals between 2 and 80 min following injection. Urine was collected from a suprapubic bladder cannula at 10-min intervals. The radioactivity of the separated plasma and of the urine was compared with a 1/200 dilution of the injection fluid. The plasma volume was measured using Evans blue, and the packed cell volume determined with a microhaematocrit.

The same procedure was carried out on another group of 70 rats (weighing 137 to 222 g), except that these rats were infused with mannitol solution containing synthetic 5-valine angiotensin II amide (Hypertensin; Ciba) at varying concentrations, equivalent to 0,3 to 2,5 ng/min/g body weight; in 36 of these rats, the carotid arterial pressure was recorded with a Grass strain gauge manometer.

Twenty-three rats were given a diet containing 4% protein for 75 days after weaning. This diet was identical to that recommended by Stead and Brock,[6] except that 50% of the dextran was replaced by sucrose, and the cod liver oil was omitted. These rats, and another 21 of similar weight on a standard diet of rat cubes (22% protein), were studied in the same way, except that no mannitol infusion was given, and the urine was not collected.

Eight unanaesthetised rats (weighing 133 to 180 g) were restrained in a wire mesh tube. Heparin (1 unit/g) was injected through a polyethylene cannula, which had been placed in the jugular vein 7 days previously. Ten minutes later, ^{51}Cr-EDTA was rapidly (3 sec) injected through the same cannula, and 50-μl blood samples were collected from the cut end of the warmed tail tip at intervals between 2 and 80 min after injection. Seven days later, the procedure was repeated after anaesthesia with pentobarbitone (40 μg/g).

A semilogarithmic plot of the disappearance curves for thiosulphate and radioactivity was constructed for each animal, using parameters calculated by the method of least squares (Dr G. T. W. Blake's computer programme). Each disappearance curve showed two exponential components

so that the volume of distribution could be calculated using the 2-compartment model.[7] The volume of distribution of thiosulphate was also calculated from the final exponential slope of its disappearance curve.[5] The plasma clearance after a rapid intravenous injection was calculated: clearance in ml/min = R/A, where R is the quantity injected, and A is the time integral of the disappearance curve,[8] and can be calculated from the fitted parameters of the disappearance curve.[7]

The rat's surface area was calculated:

area in cm^2 = 13,2 (body weight in g)^{0,64}.[9]

The difference between groups was tested by Wilcoxon's and by Kruskal-Wallis's methods for ranked data as appropriate.

RESULTS

Rabbit Experiments

Fig. 1 shows that there were 2 exponential components to the semilogarithmic disappearance curve for both thiosulphate and ^{51}Cr-EDTA, so that the volume of distribution corresponds to a 2-compartment model.[7] The volume of distribution ($\overline{V}$), calculated from the final exponential slope of the thiosulphate disappearance curve, assuming a single-compartment model, was significantly greater ($P<0,04$) than the total volume of distribution ($\overline{V}_1 + \overline{V}_2$) calculated from both the initial and final slopes of the disappearance curve: + 19% (+12 to + 75), median and range. Although the median total volume of distribution ($\overline{V}_1 + \overline{V}_2$) of thiosulphate was 17% greater than for ^{51}Cr-EDTA, the difference was not significant, median: 17 cf 14,5 ml/100 g body weight. The median plasma clearance of thiosulphate was 40% greater than for ^{51}Cr-EDTA (P = 0,063), median : 3,5 cf 2,5 ml/min/kg body weight.

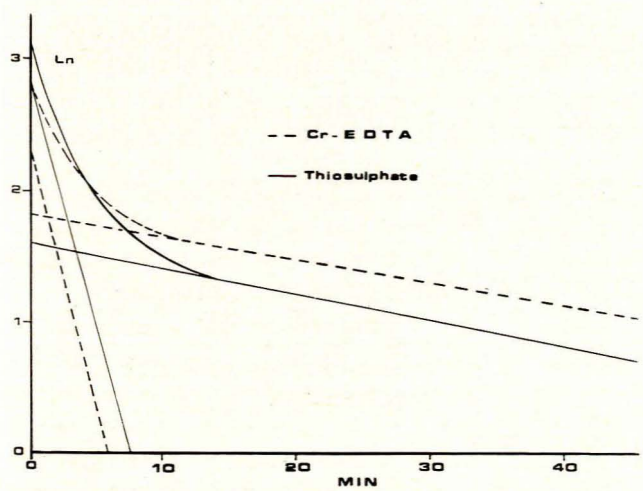

Fig. 1. A semilogarithmic graph of the plasma thiosulphate concentration and radioactivity expressed as a fraction of the injected dose per gram body weight (i.e. in g/ml *v.* time in min).

Infused Rats

The correlation of logarithmic plasma clearance of ^{51}Cr-EDTA with logarithmic body weight was + 0,83, $P<$ 0,001, with the following regression equation:

Clearance in ml/min = 0,041 (body weight in g)^{0,64}.

This suggests that the GFR is proportional to the body surface and should be expressed as ml/min/m^2 for purposes of comparison.

Both the ECF volume (calculated as the total volume of distribution of ^{51}Cr-EDTA) and the plasma volume were closely correlated with body weight and can most conveniently be expressed as ml/g of body weight.

ECF volume in ml = 0,27 (body weight in g)^{0,97}, r = + 0,81

or ECF volume in ml = 0,21 (body weight in g) + 5,2, r = + 0,64.

Plasma volume in ml = 0,065 (body weight in g)^{0,92}, r = + 0,63,

or plasma volume in ml = 0,036 (body weight in g) + 0,69, r = + 0,74.

The initial volume of distribution ($\overline{V}_1$) in the 2-compartment model was found to be some 3 times larger than the plasma volume, and represented about half of the total volume of distribution ($\overline{V}_1 + \overline{V}_2$).

The plasma clearance of ^{51}Cr-EDTA calculated from the urinary excretion/geometric mean plasma concentration, was significantly less ($P<0,01$) than the clearance calculated from the disappearance curve: −15% (−64 to +20) median, 10th and 90th percentiles.

The plasma clearance of ^{51}Cr-EDTA calculated from the urinary excretion was significantly less ($P<0,01$) in the last 10-min urine collection period, than in the second 10-min period: −10% (−25 to +12), median, 10th and 90th percentiles.

Angiotensin Infusion

Table I shows that the GFR calculated from the plasma clearance of ^{51}Cr-EDTA was significantly reduced during angiotensin infusion, and it was found that there was a small but significant negative correlation between the GFR and the rate of angiotensin infusion from 0 to 2,5 ng/min/g: r = −0,29; $P<0,01$.

TABLE I. GFR, URINE VOLUME AND RISE IN ARTERIAL PRESSURE DURING AN INFUSION OF ANGIOTENSIN

	Angiotensin (ng/g/min)		
	Nil	0,3 to 1,4	1,5 to 2,5
GFR (ml/m^2/min)	31* (26 to 44)	26 (15 to 37)	27 (20 to 37)
Urine volume (µl/min)	100 (66 to 185)	64 (39 to 145)	185 (83 to 287)
Blood pressure (mmHg)	—	+20 (0 to 40)	+37 (18 to 65)

Median is above the 10th and 90th percentiles in brackets.
* $P<0,02$ compared with both angiotensin groups.

Table I also shows that the volume of urine was reduced during angiotensin infusions at 0,3 to 1,4 ng/min/g body weight, but increased when 1,5 to 2,5 ng/min/g was infused. There was a highly significant quadratic curvilinear regression of urine volume on the rate of angiotensin infusion from 0 to 2,5 ng/min/g ($F = 23$, $P<0,001$). The regression line and its 95% confidence limits are shown in Fig. 2, and it can be seen that the minimum urine flow corresponds to an infusion of 0,9 ng/min/g (95% confidence limits 0,4 to 1,8 ng/min/g). The urine flow was not correlated with the GFR or with the rise in blood pressure.

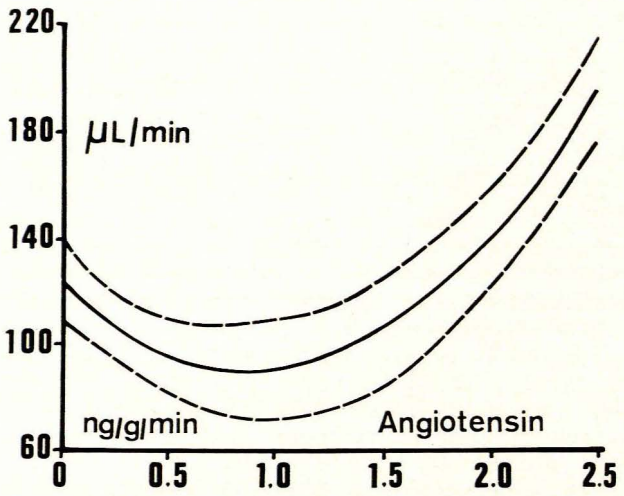

Fig. 2. The rate of urine flow in µl/min at each rate of angiotensin infusion from 0 to 2,5 ng/g/min. The quadratic regression line is shown with its 95% confidence limits.

Anaesthesia

Pentobarbitone reduced the plasma clearance of ^{51}Cr-EDTA by −15% (−37 to +24 range), but the difference was not significant.

Protein Energy Malnutrition

Ten of the 23 rats developed subcutaneous oedema, particularly on the ventral surface of the neck. Table II shows that there was no difference in GFR (calculated from the plasma clearance of ^{51}Cr-EDTA) between the malnourished rats and rats of similar weight fed on a standard diet. On the other hand, the ECF volume (calculated from the total volume of distribution of ^{51}Cr-EDTA) was significantly increased in the oedematous rats, and their packed cell volume (PCV) was significantly reduced.

TABLE II. ECF VOLUME, GFR, PCV AND BODY WEIGHT OF RATS ON 4% AND 22% PROTEIN DIETS

	22%	4% Oedema	4% No oedema
ECF volume‡ (ml/100 g)	24* (15 to 31)	30 (25 to 45)	22 (20 to 44)
GFR (ml/m²/min)	24 (16 to 36)	29 (12 to 39)	25 (9 to 42)
PCV (%)	43† (37 to 48)	19 (11 to 37)	37 (25 to 43)
Body weight (g)	71 (44 to 79)	69 (59 to 88)	61 (58 to 75)

* $P<0,25$ compared with both groups of rats on 4% diet.
† $P<0,01$ compared with both groups of rats on 4% diet.
‡ $P<0,02$ comparing all 3 groups.

DISCUSSION

The volume of distribution ($\overline{V}$) of thiosulphate calculated from the final exponential slope of the disappearance curve (after a rapid intravenous injection), overestimates the ECF volume. It would seem preferable to calculate the total volume ($\overline{V}_1 + \overline{V}_2$) from both exponential components of the curve, rather than to attempt to compensate by giving an arbitrarily slower injection.[10] There is a considerable extrarenal clearance of thiosulphate so that it is unsuitable for the measurement of the GFR;[5,10] it was also found that in the rat a small proportion of ^{51}Cr-EDTA was not cleared into the urine, so that the plasma clearance may also overestimate the true GFR, as has previously been reported for man.[11] In the rabbit, the total volume of distribution of ^{51}Cr-EDTA appears to be a satisfactory measure of the ECF volume, and its determination is technically more simple and accurate than for the standard thiosulphate method.

In the oedematous, protein-deficient rat, the ECF volume was found to be expanded, although no change in the GFR could be detected. In agreement with previous studies on the rat[12-14] it was found that angiotensin produced a small, but significant, decrease in GFR, and that in the lower doses (0,3 to 1,4 ng/min/g), there was a simultaneous decrease in the rate of urine formation, whereas larger amounts of angiotensin produced a diuresis.

It would seem possible, therefore, that in the rat the increased activity of the renin-angiotensin system associated with protein energy malnutrition[15,16] could lead to fluid retention and an expansion of the ECF volume by opposing the excretion of urine by the kidneys.

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