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EFFECT OF THE PROTEIN CONTENT OF THE DIET ON THE GLOMERULAR FILTRATION RATE OF YOUNG AND ADULT RATS

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It has been shown (Dicker & Heller, 1945) that the kidney function of adult rats, fed on a standard diet, resembled that of the dog, i.e. the rate of glomerular filtration in rats is independent of that of urine flow. In rats fed on a protein-deficient vegetable diet, however, and suffering from severe hypoproteinaemia, the glomerular filtration rate was found to vary with the urine flow (Dicker, Heller & Hewer, 1946). An investigation of the kidney function of normal rats on diets containing different amounts of protein and thus producing different plasma-protein concentrations seemed therefore of interest.

METHODS

Experimental animals. Twenty-nine young rats with weights varying from 101 to 150 g. and ninety-one adult rats with weights from 260 to 350 g. were used.

Diets. The animals were fed on different types of diet, yielding comparable amounts of calories but containing varying amounts of protein.

Diet I (D. I) had the following composition: wheat offal 19·2 %, ground wheat 19·2 %, finely ground oats 19·2 %, ground maize 9·5 %, ground barley 9·5 %, white fish meal 4·8 %, meat and bone meal 9·5 %, dried skimmed milk 7·0 %, dried yeast 1·3 %, salt 0·4 % and cod-liver oil 0·4 %. 100 g. of this food yielded 305·8 calories. Diet II (D. II) differed from D. I by its higher content of dried skimmed milk, viz. 14 % instead of 7 %. Its composition was so adjusted as to have the same calorific value as D. I. Diet III (D. III) had the following composition: casein 18 %, starch 53 %, dried yeast 15·0 %, ground-nut oil 8·0 %, cod-liver oil 2·0 %, salt mixture 4·0 %. 100 g. of this diet yielded 303·0 calories. Diet IV (D. IV) differed from D. III by its higher content of casein, viz. 25·0 % instead of 18 %. Litter-mates were fed on diet D. II until they reached the required weight (101–150 g.). Series of adult rats were fed on the different diets described for 9 weeks.

Experimental procedures for the determination of inulin clearances. The routine procedure conformed on the whole with that described previously (Dicker & Heller, 1945) but differed in the following respect: 1·0 ml./100 g. body weight of a 5 % solution of inulin in physiological saline was injected intramuscularly, followed immediately by the administration of 5 % of body weight of water by stomach tube. Fifty minutes after water administration the bladder was emptied and the urine-collecting period started; the latter varied between 10 and 15 min. according to the urine flow of the rat. The equilibrium reached 50 min. after the injection was satisfactory: the level of inulin in the plasma did not vary significantly during the period of urine collection. Immediately after the end of the collecting period, the rats were anaesthetized and blood, obtained from the carotid and jugular vessels, was mixed with heparin.

Analytical methods. Inulin in plasma and urine were determined by the method of Smith, Goldring & Chasis (1938). The inulin used was that of Hopkin and Williams Ltd. Plasma-protein concentrations were estimated by the copper sulphate method for measuring specific gravities (Phillips, Van Slyke, Dole, Emerson, Hamilton & Archibald, 1945); Hoch & Marrack's (1945) formula was used for the calculation of the concentration of plasma protein (Dicker, 1948).

Methods of calculation. To permit comparison with previous results (Dicker & Heller, 1945) inulin clearances (=rate of glomerular filtration=G.F.R.) were expressed in ml./100 g. body weight/min.

Statistical treatment of results. Results are given as means and standard error of the mean. The correlation coefficient 'r' was calculated according to Mainland (1938); the probability (P) for 'r' was obtained from the tables of Fisher & Yates (1943). The standard error of 'r' was calculated as $= \pm(1-r^2)/\sqrt{n}$. The coefficient of regression (b) and the regression lines were calculated according to Bradford Hill (1942); the standard error of 'b' was calculated as S.E. $= \pm \sigma/r \times b$ (Pütter, 1929).

RESULTS

In spite of the difference in the composition of the diets given to the rats, they all looked healthy; the increase of weight in adult rats, however, though significant, was not the same in each series; it was least marked in rats fed on diet D. I, in which the average increase of weight in 62 days amounted to 24·0% of the initial weight as compared with 60·0% in rats fed for the same period on diet D. IV (Table 1). Differences in the concentration of plasma

TABLE 1. Effects of protein content of the diet on kidney weight and glomerular filtration rate

	Young rats (29)	Adult rats (21)	Adult rats (25)	Adult rats (22)	Adult rats (23)
Type of diet	D. II	D. I	D. II	D. III	D. IV
Body wt. (g.)	101·0–137·0	265·0–325·0	270·0–345·0	265·0–350·0	260·0–330·0
Increase of wt. in 9 weeks (%)	—	24·0	30·0	45·0	60
Plasma proteins (g./100 ml.)	5·23±0·087	5·84±0·113	6·44±0·047	6·83±0·026	7·33±0·026
Kidney wt. (g.)	1·42±0·082	2·05±0·082	2·08±0·057	2·21±0·085	2·48±0·069
Kidney wt./100 g. body wt.	1·24±0·048	0·75±0·024	0·79±0·052	0·82±0·022	0·88±0·059
G.F.R. (ml./100 g./min.)	Correlated with urine flow			0·43±0·009	0·76±0·335
Coeff. of regression between G.F.R. and urine flow ('b')	+10·0 ±0·54	+8·7 ±0·21	+3·9 ±0·83	−1·4 ±1·09	−0·9 ±2·88

The figures for plasma proteins, kidney weight, kidney weight/100 g. body weight and G.F.R. are mean results with their standard error. 'b' = coefficient of regression and standard error. Number of experiments in parentheses.

proteins, in the kidney weights and in the kidney weight/100 g. body weight ratio of each series were also noticed. Table 1 shows that in young and adult rats kidney weight varied directly with the concentration of the plasma proteins, and that in adult rats the kidney weight/100 g. body weight varied with the type of diet and thus with the plasma-protein concentration.

Results of inulin clearances estimations are given in Fig. 1. They show that in young rats (body weight 101·0–150·0 g.) and in adult rats fed on diets with a low protein content (D. I and D. II), the rate of glomerular filtration was correlated with the urine flow: $b = +10{\cdot}0$, s.e. $\pm 0{\cdot}54$ and $b = +8{\cdot}7$, s.e. $\pm 0{\cdot}41$, respectively. The correlation between G.F.R. and urine flow was still significant

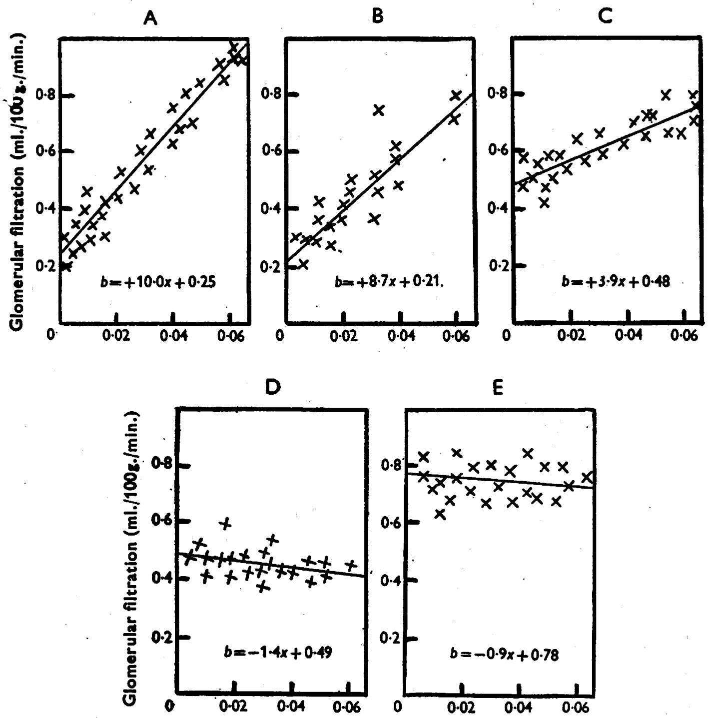

Fig. 1. Effect of protein content of the diet on the glomerular filtration rate of young and adult rats during a water diuresis. A, young rats fed on a diet containing 14% dried skimmed milk; B, adult rats fed on a diet containing 7% dried skimmed milk; C, adult rats fed on a diet containing 14% dried skimmed milk; D, adult rats fed on a diet containing 18% casein; and E, adult rats fed on a diet containing 25% casein. The differences in the slope of the regression line (see also equations) will be noted.

in rats fed on diet D. II: $b = 3{\cdot}9$, s.e. $\pm 0{\cdot}83$. But in rats fed on a diet containing 18% of casein (D. III) the rate of glomerular filtration was independent of that of urine flow (Table 1). The mean value for G.F.R. amounted to $0{\cdot}43 \pm 0{\cdot}009$ (s.e. of mean of twenty-two observations) ml./100 g. body weight/min., a figure that compares with previous results obtained with adult rats fed with the same type of diet (Dicker & Heller, 1945). By increasing the amount of protein in the food (D. IV) and concurrently that in the plasma, the rate of glomerular filtration was significantly increased but remained independent of the rate of urine flow (Table 1). Its mean value was 0·76 ± 0·335 (23) ml./100 g. body weight/min., a value comparable with that found by Lippman (1947), and by Friedman, Polley & Friedman (1947).

DISCUSSION

Recent work on kidney function in rats conveys the impression of a lack of agreement with reference to (*a*) the relation between the rate of glomerular filtration and that of urine flow, and (*b*) the mean value of the inulin clearances (= glomerular filtration rate). Some workers (Dicker & Heller, 1945; Friedman *et al.* 1947; Lippman, 1947) found that the inulin clearances in adult rats were independent of the rate of urine flow, thus resembling in this respect adult man and the dog (Smith, 1937); others (Braun Menendez & Chiodi, 1946; Friedman, 1947) claimed that the inulin clearances varied directly with the urine flow, as in rabbits (Kaplan & Smith, 1935; Dicker & Heller, 1945; Forster, 1947). Further confusion arose from the fact that some workers (Dicker & Heller, 1945; Friedman *et al.* 1947) related the mean values of the inulin clearance to body weight, others (Braun Menendez & Chiodi, 1946) to body surface and others again (Lippman, 1947) to kidney weight.

Kidney weight is accepted to be related to body weight and surface area and is usually calculated from body weight, hence the choice of the parameters would seem to be immaterial. But, as it could be shown that the kidney weight/100 g. body weight varied (*a*) with the age of the rat and (*b*) with the type of diet given to the animal, it follows that none of the proposed parameters (body weight, kidney weight or surface) can be relied upon unless the type of experimental animal and the diet have been standardized.

The importance of standardization is also borne out by the lack of agreement in the results of the inulin clearances estimated by different workers. Braun Menendez & Chiodi (1946) used eighty-four rats with a weight varying from 121·0 to 430·0 g., thirty of which weighed less than 165·0 g.; these authors found that there was a very close correlation between the rate of glomerular filtration and that of urine flow ($r = +0{\cdot}87$). If the series of Braun Menendez & Chiodi (1946) were divided into two groups, one of rats with a weight varying from 121·0 to 200·0 g. and another of rats with a body weight from 201·0 to 430·0 g., it could be calculated from their data that the correlation between the rate of glomerular filtration and urine flow in the first group was highly significant, but only just significant in the second ($r = +0{\cdot}33$; $P < 0{\cdot}05 > 0{\cdot}02$). This suggests that the first group compares with the 'young rats' of the present series and the second group with the more adult rats of the present series which were fed on a diet which had a low protein content.

A last point to be considered is the rate of the glomerular filtration. Shannon (1942) has shown that the administration to dogs of a saline solution (85 mmol. NaCl) increased significantly the rate of the glomerular filtration; the same observation has been made in rats (Dicker, 1946). Furthermore, Ayer, Schiess & Pitts (1947) have shown that in dogs fed on a meat diet the rate of glomerular filtration could increase by nearly 100% when compared with dogs fed on a carbohydrate diet. A similar finding was made in rats; it could be shown that by varying the amount of casein in the diet from 18·0 to 25·0%, the mean inulin clearance (=glomerular filtration rate) could be increased from 0·43 to 0·76 ml./100 g. body weight/min.

It is thus clear that provided the animal material is standardized, with reference to age and to diet, good agreement will be found in estimating the kidney function in rats.

SUMMARY

1. Inulin clearances (=glomerular filtration rate) were estimated (*a*) in young rats with weights ranging from 101·0 to 150·0 g., (*b*) in adult rats with weights varying from 260·0 to 350·0 g.

2. The plasma-protein concentration of the adult rats varied with the amount of protein in the diet.

3. In young and in adult rats with a plasma-protein concentration below 6·80 g./100 ml., the rate of glomerular filtration was correlated with that of the urine flow.

4. In adult rats fed on a diet containing at least 18% casein, and having a plasma-protein concentration above 6·80 g./100 ml., the rate of glomerular filtration was independent of that of the urine flow.

5. High concentrations of protein in plasma were accompanied by higher mean rates of glomerular filtration.

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