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THE MECHANISM OF WATER DIURESIS IN ADULT AND NEWBORN GUINEA-PIGS

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Recent work in renal physiology has emphasized species differences in mammalian kidney function. It seemed, therefore, of interest to investigate the kidney of the guinea-pig which, in this respect, has so far been neglected. All that appears to be known is that the time relations of water diuresis in adult guinea-pigs resemble those of the rat rather than those of the rabbit (Heller & Smirk, 1932). This observation suggested further analysis of renal function by means of clearance estimations. The work was extended to newborn guinea-pigs.

METHODS

Experimental animals

Sixty-six adult guinea-pigs (mean weight 450 g.) kept in runs in the open air up to the time of the experiment and sixty-seven guinea-pigs of the same strain, 20–30 hr. old (mean weight 70 g.) were used.

Inulin clearances in adult guinea-pigs

Adult guinea-pigs were given 5·0 ml./100 g. body weight of tepid water by stomach tube and were then injected subcutaneously with 2·0 ml./100 g. body weight of a 5% solution of inulin. Handling, prodding or even suprapubic pressure failed to induce emptying of the bladder; it was therefore necessary to rely on spontaneous micturition. Spontaneous emptying of the bladder occurred readily after water administration. The voiding of the bladder which occurred 90 to 105 min. after the water had been given, i.e. during the peak of the water diuresis (Heller & Smirk, 1932) was taken as the beginning of the urine collecting period. A second micturition (=end of the urine collecting period) occurred spontaneously between 14 and 27 min. after the first. The average time of collecting period amounted to $20{\cdot}0 \pm 1{\cdot}7$ min. (S.E. of nineteen observations). Immediately after the second micturition the animal was killed and blood collected from the jugular and carotid vessels. Heparinized plasma was used throughout.

Inulin excretion by newborn guinea-pigs

Newborn guinea-pigs cannot be made to empty their bladder at regular intervals, and even after spontaneous micturition their bladder is not quite empty. To collect urine quantitatively the newborn animals had to be killed and the urine withdrawn by bladder puncture. Short urine collecting periods for the estimations of inulin clearances were thus impossible. To overcome this difficulty, the following method was used: newborn guinea-pigs were given 5·0 ml./100 g. body weight of tepid tap water by stomach tube and injected subcutaneously with 2·0 ml./100 g. of

a 5% solution of inulin. Immediately after the injection, they were put in individual metabolism cages and kept for 5 hr. at room temperature. At the end of the period, they were killed by decapitation, blood collected in heparinized tubes and the urine withdrawn by bladder puncture. The bladder urine was added to any urine which had been spontaneously excreted during the period of observation. Inulin was estimated in plasma and in urine. A series of adult controls were treated in an identical manner except that the urine collecting period was started immediately after the animal had emptied its bladder spontaneously.

Calculation of true inulin concentration in the urine of newborn animals

As newborn guinea-pigs do not empty their bladder completely, there was always a certain amount of residual urine in their bladder before they were injected with inulin. To calculate the true concentration of inulin in the urine collected during the 5 hr. of observation, the following formula was used:

$$C_A = \frac{C_0 \times V}{V - V_r}, \quad (1)$$

where C_A = true concentration of inulin in urine (mg./100 ml.), C_0 = the estimated concentration of inulin (mg./100 ml.), V_r = volume of residual urine (ml./100 g. body weight) found in the bladder of a control series of newborn guinea-pigs killed just before the beginning of the experiment and V = total urine volume (ml./100 g. body weight) collected at the end of the experiment.

Water excretion in adult and newborn guinea-pigs

(*a*) *Dilution test.* Adult and newborn guinea-pigs were given an amount of water equal to 5% of their body weight by stomach tube, and were put singly into glass metabolism cages for 3 hr. Each time that adult guinea-pigs voided urine spontaneously its volume and specific gravity were measured. The newborn animals were killed at intervals of 60 min. and the urine withdrawn by bladder puncture. The volume of residual urine in similar newborn animals was taken into account to calculate the true urinary volume and its specific gravity. There was no residual urine in the adult animal as experiments on them were started only when the animal had emptied its bladder spontaneously.

(*b*) *Concentration test.* Adult and newborn guinea-pigs kept in individual metabolism cages were deprived of food and water for 24 hr. In the adults, the specific gravity of samples collected after 8 and 24 hr. was measured. In the newborn guinea-pigs, urine samples were collected as they were voided, except the last sample which was withdrawn by bladder puncture after the animal had been killed. Each sample was measured and its specific gravity estimated.

Determination of specific gravity of urine

Heller's (1940) apparatus was used. To estimate the true specific gravity of the urine samples, an equation similar to that for the calculation of true concentration of inulin was used (Heller, 1949).

Analytical method

Inulin in plasma and urine was determined by the method of Smith, Goldring & Chasis (1938).

RESULTS

Water diuresis in adult and newborn guinea-pigs

Fig. 1 shows the rate of urinary output following the administration of 5·0 ml. water/100 g. body weight. The results in the adult animals confirm those of Heller & Smirk (1932). During water diuresis, the specific gravity of their urine decreased to $1{\cdot}0083 \pm 0{\cdot}0002$ (12).

The residual urine, i.e. that present in the bladder before administration of water in a series of newborn guinea-pigs amounted to $0{\cdot}95 \pm 0{\cdot}052$ (12) ml./100 g. body weight. Another series of newborn guinea-pigs were given 5·0 ml./100 g.

body weight of water by stomach tube and were killed after 60, 120 and 180 min. To calculate the percentage of administered water excreted, the mean value of residual urine was subtracted from the amounts of urine found in the bladder. No spontaneous micturition occurred in any of these experiments. It will be seen from Fig. 1 that while adult guinea-pigs excreted $116{\cdot}9 \pm 6{\cdot}18$ (12) % of the administered water in 3 hr., newborn guinea-pigs excreted $52{\cdot}2 \pm 4{\cdot}60$ (12) % only. The mean specific gravity of urine samples of newborn guinea-pigs collected during water diuresis amounted to $1{\cdot}0092 \pm 0{\cdot}0006$ (12) and was thus not significantly different from those of the adults.

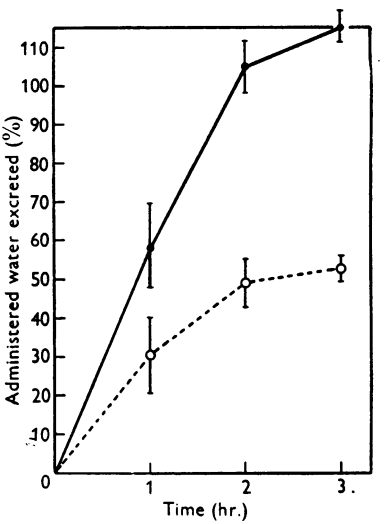

Fig. 1. Comparison between the urinary output of adult and newborn guinea-pigs after administration of 5 ml. water/100 g. body weight. ●——● = mean urinary output of adult animals; ○- - - -○ = mean urinary output of newborn animals. The vertical lines indicate the standard error.

Effect of dehydration on urinary excretion

(*a*) *Adult guinea-pigs.* The animals were kept in individual metabolism cages, at room temperature (19–20° C.) and food and water were withheld for 24 hr.: the specific gravity was estimated in the urine collected during the last 16 hr. of observation. The animals were killed at the end of the period of experimentation; any bladder urine found was measured and its specific gravity estimated. The results suggest that the ability of adult guinea-pigs to concentrate urine is much less marked than in adult rats (Heller, 1949), the maximum concentration observed under the same conditions being $1{\cdot}056 \pm 0{\cdot}0022$ (20) in rats and $1{\cdot}026 \pm 0{\cdot}0016$ (12) in guinea-pigs.

(*b*) *Newborn guinea-pigs.* It proved impossible to induce micturition in newborn guinea-pigs. The specific gravity of the urine in the dehydrated animals was therefore usually determined in 24 hr. urine samples using urine withdrawn

by bladder puncture. In some cases, however, newborn animals voided urine spontaneously during the first 8 hr. of the period of dehydration. The bladder urine collected from these animals was therefore more nearly comparable to that obtained from the dehydrated adult guinea-pigs; its specific gravity amounted to 1·026 ± 0·0013 (12); there was thus no significant difference between the specific gravity of urine samples of dehydrated adult and of newborn guinea-pigs.

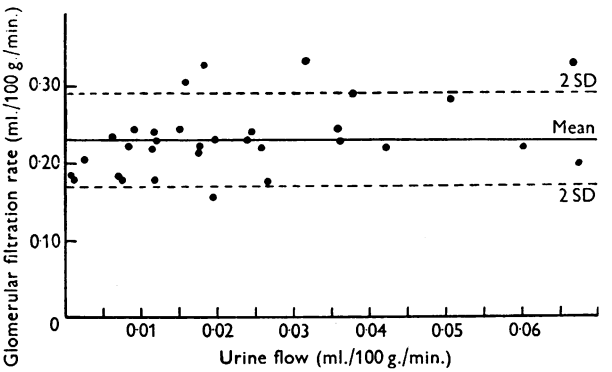

Fig. 2. Relation between inulin clearance (=glomerular filtration rate) and rate of urine flow in adult guinea-pigs.

Inulin clearance (glomerular filtration rate) in adult guinea-pigs

The glomerular filtration rate of adult guinea-pigs as estimated from inulin clearances amounted to a mean of 0·23 ± 0·006 (32) ml./100 g. body weight/min.; it did not vary with the rate of urine flow (Fig. 2).

Rate of inulin excretion in newborn and adult guinea-pigs

The amount of inulin excreted in 5 hr. by newborn guinea-pigs, to which the standard dose of water and of inulin had been administered, was compared with that excreted by adults treated in an identical manner. Fig. 3 shows that while in adult guinea-pigs the amount of inulin excreted per 100 g./min. was independent of the urine flow, the rate of inulin excretion in the newborns varied with the rate of urine flow. At higher rates of urine formation the rate of inulin excretion by newborn guinea-pigs reached that of adults (Fig. 3). The volume of the urine withdrawn from the bladder of the newborns represented the sum of residual urine and urine excreted during the experimental period, but the true concentration of inulin in the urine was calculated by means of equation (1) (see Methods). Relating this value to the plasma inulin concentration found when the animals were killed, it was possible to obtain some indication of the rate of tubular water reabsorption at different urine outputs. When comparing such values for the inulin U/P ratio in newborn guinea-pigs with inulin U/P ratios obtained in adult animals by the same procedure, it will be

seen (Fig. 4) that the rate of tubular water reabsorption at comparable rates of urine flow appeared to be much the same in the two series. These results would agree with the fact that during dehydration and hydration the same values for the specific gravity of urine was observed in newborn and adult guinea-pigs.

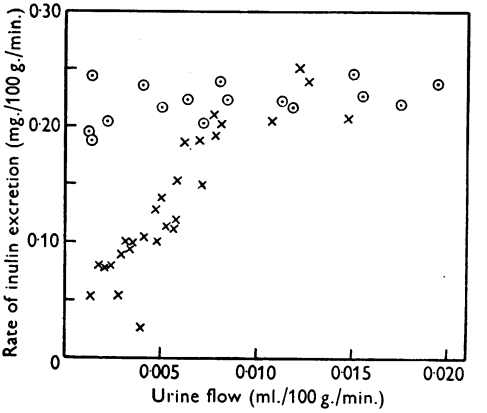

Fig. 3.

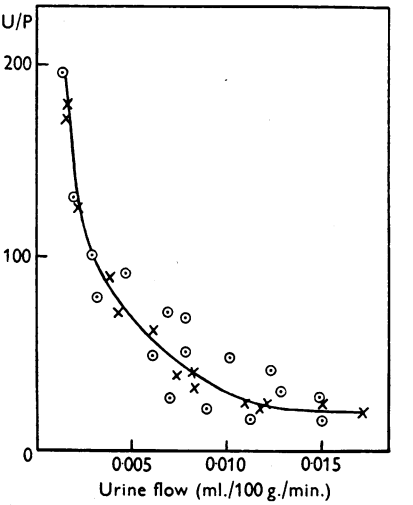

Fig. 4.

Fig. 3. Relation between rate of inulin excretion and rate of urine flow in newborn and adult guinea-pigs. × =newborn animals; ⊙ =adult animals.

Fig. 4. U/P ratio of inulin in newborn and adult guinea-pigs related to rate of urine flow. × =adult animals. ⊙ =newborn animals.

DISCUSSION

It is a puzzling fact that during water diuresis the rate of glomerular filtration and that of urine flow are correlated in the rabbit (Kaplan & Smith, 1935; Wilkinson & McCance, 1940; Dicker & Heller, 1945; Forster, 1947), while no such correlation has been found in another species of rodent, viz. the adult rat fed on an adequate diet (Dicker & Heller, 1945; Dicker, 1949). As shown recently by Dicker & Heller (1950), the glomerular component in the regulation of the urine volume in rabbits appears to be a physiological response in this species. The experiments on adult guinea-pigs, reported in this paper, show that renal function in this rodent resembles that of the rat rather than that of the rabbit, i.e. the urine volume during a water diuresis is regulated by tubular water reabsorption exclusively. Our results suggest further that the differences in renal function between rabbit and guinea-pig cannot be explained by their nutritional habits as both species are herbivorous.

All available criteria indicate that guinea-pigs are more mature at birth than the newborn of other laboratory mammals and man: newborn guinea-pigs show co-ordinated locomotion and position reflexes, they are not blind and their

incisors and molars are present at birth. Furthermore, there are indications that the tissue metabolism of newborn guinea-pigs is more mature: their tolerance to anoxia has been shown by Fazekas, Alexander & Himwich (1941) to be lower than that of the newborn cat, dog, rabbit or rat; in fact, according to Cheymol (1948), it is no greater than that of the adult. It is therefore not surprising that the renal function of newborn guinea-pigs compared in many respects with that of adult animals. Dilution and concentration tests showed much the same values for urinary specific gravity in both adults and newborns. Inulin U/P ratios also were much the same at comparable rates of urine flow, suggesting mature function of the renal tubules in the newborn guinea-pig. There seem, however, to be differences in glomerular function: after the administration of water to newborn animals the glomerular filtration rate increased with the rate of urine flow; at low rates of urine flow the rate of glomerular filtration was much below that encountered in adult animals, but it reached the adult level at high rates of urine flow. The newborn guinea-pigs excreted a smaller fraction of a standard dose of administered water in a given time than adults, as do puppies (Adolph, 1943) and newborn rats (Heller, 1947; McCance & Wilkinson, 1947). It is conceivable that the variability of the glomerular filtration rate observed has a bearing on this finding, though extra-renal factors cannot be excluded.

SUMMARY

1. Inulin clearances in adult guinea-pigs did not change significantly with variations of urine flow ranging from 0·001 to 0·065 ml./100 g. body weight/min. The mean inulin clearance amounted to $0{\cdot}23 \pm 0{\cdot}006$ (32) ml./100 g. body weight/min.

2. Determination of rates of inulin excretion in newborn guinea-pigs showed that glomerular filtration rate increased with increasing urine flow.

3. Adult guinea-pigs excreted $116{\cdot}9 \pm 6{\cdot}18$ (12)% of a standard dose of administered water in 3 hr., while newborn animals excreted $52{\cdot}2 \pm 4{\cdot}61$ (12)% only, suggesting that water diuresis was incompletely developed in guinea-pigs aged approx. 24 hr.

4. Specific gravity estimation of urine samples and determinations of inulin U/P ratios during water diuresis showed that the urine of newborn guinea-pigs becomes diluted to much the same extent as that of adults. The ability of newborn animals to concentrate was similar to that of adult guinea-pigs.

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