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THE RESPONSE OF ADULT AND SUCKLING RATS TO THE ADMINISTRATION OF WATER AND OF HYPERTONIC SOLUTIONS OF UREA AND SALT

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The work which has been done in the last few years has emphasized the fact that hypertonic solutions of chlorides and of urea provoke a large diuresis in dehydrated subjects (Adolph, 1921; Hervey, McCance & Tayler, 1946*a*, *b*). Most of the work in this country has been done on adult human subjects and in America on adult dogs (Elkinton & Taffel, 1942), but there is evidence in the literature that, for some time after birth, the kidney may not function as it does in adult life. The work now to be reported was undertaken (*a*) to study the effects of hypertonic solutions upon dehydrated adult animals, (*b*) to compare the effects of treating newborn animals in a similar way. In connexion with this the responses of adult and suckling animals to comparable amounts of water have also been investigated. Work on animals other than rats will be reported later.

METHODS

Animals. In the experiments on adult animals the rats used were of both sexes. They were 6 months old; the females weighed about 250 g. and the males about 350 g. Throughout the experiments they were maintained on a standard stock diet. The young animals were taken away from their mothers as soon as the last member of the litter had been born. They were unfed. Throughout the experiment they were kept in a box lined with cotton-wool and warmed with an electric bulb to about 30° C. Ten or more were used for each experiment.

Urine collections. Food and water were removed from the cages of the adult rats 18 hr. before the beginning of an experiment. The bladders of the animals were emptied before and at approximately hourly intervals during each experiment. This was done by gently compressing the abdomen, and, with practice, it was possible to feel how full their bladders were and when they were empty. The rats were kept during the experiments in aluminium metabolism cages resting on glass funnels, and the urine was collected under toluene in finely graduated cylinders of 10 c.c. capacity. At the end of each collecting period the urine expressed manually was added to the amount which had collected in the cylinder.

The urine from the newborn rats was collected in small weighed bottles. At the beginning of the experiment the bladders were emptied by compression and gentle stroking of the perineum. It seems that in the early stages of life these animals do not empty their bladders spontaneously, but depend upon a reflex initiated by the mothers licking the perineum. The emptying of the bladders was repeated at the end of each collecting period.

Administration of solutions. The adult rats received all solutions by stomach tube. The finest commercial rubber tubing was too thick to pass into the stomach of the newborn rats, but it was possible to remove the insulation tubing from the finest electrical wire, and to pass this down into the stomach of the sucklings. Most solutions were given by this means, but some were administered intraperitoneally to compare the degree of absorption from the intestine and the peritoneal cavity. Both adult and suckling animals received comparable amounts of the solutions. Usually 5% of the body weight was given.

Collection of blood. Two samples of blood were taken in each experiment. Blood was obtained from the tips of the tails of the adult animals and collected in heparinized tubes under paraffin. It was centrifuged immediately and the plasma separated. The first sample was taken in the middle of the preliminary (control) period, before the experimental solution had been administered, and the second in the middle of the period during which the peak of the diuresis was expected to fall. Blood was obtained from the newborn animals by snipping off their heads. As a result, the samples of blood had to be taken at the ends of the control and experimental periods. Half the newborn rats in each experiment were, therefore, killed at the end of the preliminary collection of urine, and the remainder at the end of the experiment. The blood was collected under paraffin in heparinized 'Durham' tubes which were centrifuged at once to separate the plasma.

Chemical estimations. Urea was estimated in blood and urine by the xanthydrol method (Lee & Widdowson, 1937). Chlorides were estimated by the ultramicro method of Sendroy (1937). When enough urine was available, chlorides were also estimated as described by McCance (1937). The depression of the freezing point was determined either by a Beckmann thermometer or by a micro-method which required less than 0·1 c.c. urine. The difference in potential between thermocouples in the unknown solution at its freezing point and in melting ice made from distilled water was measured by means of a sensitive galvanometer and potentiometer. The freezing points of a series of standard solutions were determined by means of a Beckmann thermometer and from these a graph was plotted relating freezing point to the reading on the galvanometer scale. In the tables the results have been expressed in m.osmol./l. by calculation from the observed freezing point.

RESULTS

The response of adult and infant rats to hypertonic solutions of NaCl

The response of adult rats to hypertonic solutions of salt seems to be of the same nature as the response of human adults. The data of a typical experiment are given in Table 1. The most obvious feature of this response has been the very great diuresis. It will be observed that the volume of urine passed by rats which had been dehydrated for 18 hr. was very low, and that it had a very

TABLE 1. The response of adult rats, which had been kept without water for 18 hr., to a hypertonic solution of sodium chloride given by stomach tube

Time	Minute volume c.c./1000 g. rat	Urine: Osmotic pressure m.osmol./l.	Urine: Chloride m.equiv./l.	Urine: Urea m.mol./l.	Blood chloride m.equiv./l.
9.45–11.45 a.m.	0·003	2560	290	1220	106
11.45 a.m. 5% of the body weight 10% NaCl given					
12.0 noon–1.0 p.m.	0·069	1620	416	720	—
1.0–2.10 p.m.	0·168	1380	460	340	—
2.10–3.35 p.m.	0·198	1310	460	230	—
3.35–4.35 p.m.	0·155	1390	510	230	—
4.0 p.m. Blood	—	—	—	—	116
4.35–5.35 p.m.	0·088	1440	538	235	—
5.35–7.15 p.m.	—	1530	560	560	—

high osmotic pressure. During the first hour after the administration of the NaCl solution the minute volume increased by more than twenty times and continued to rise for the next 2 hr. Later the minute volume fell gradually, but, by the next morning, it was still higher than it had been before the experiment began, and this in spite of the progressive dehydration of the body.

The osmotic pressure of the urine always fell till the diuresis reached its peak, and then gradually rose again, but, even after 12 hr., it had not reached the level of the preliminary sample. The results suggest that, under these conditions, the osmotic pressure of the urine depends upon the minute volume (Hervey, McCance & Tayler, 1946*a*, *b*). The changes in the freezing point were due mainly to changes in the urea concentrations. With the onset of the diuresis the percentage of urea in the urine fell and by the next morning it had not regained its former level. The chloride concentration, on the other hand, rose throughout the experiment and was even higher the next morning. The clearances of both substances were greatest at the peak of the diuresis.

TABLE 2. The response of newborn rats to a hypertonic sodium chloride solution given by stomach tube

Time	Minute volume c.c/1000 g. rat	Urine: Osmotic pressure m.osmol/l.	Urine: Chloride m.equiv./l.	Urine: Urea m.mol./l.	Blood chloride m.equiv./l.
12.15–3.15 p.m.	0·035	505	101	133	108
3.15 p.m. 5% of the body weight of 10% NaCl given					
3.15–7.15 p.m.	0·067	623	155	98	—
7.20 p.m. Blood collected					130

The response of the newborn kidney to the administration of salt was very different from that of the adult. Ninety-two newborn rats were used for these experiments. The results of a typical experiment are shown in Table 2. It will be seen that the preliminary urine from the infant rats was much more dilute than the corresponding sample from the adult animals. This is an observation well authenticated in man (McCance & Young, 1941; Heller, 1944). A flow of urine with an almost fixed freezing point, irrespective of the degree of hydration of the young rat, seems to be characteristic of the early life of this animal, but this will be considered more fully in connexion with the response of newborn rats to water. After the administration of hypertonic salt solutions, either by intraperitoneal injection or by stomach tube, the blood chloride rose to much higher levels than it did in adults. Both methods of administering the chloride solution caused a very similar increase in blood chloride concentration but, in spite of it, there was no sudden diuresis as in the adult animals. Sometimes the minute volume rose slightly, but, as a rule, there was no significant change. The osmotic pressure of the urine also changed little although the alteration was in the nature of an increase and not of a decrease as in adults. Analyses of the urine for chloride and urea showed how this was brought about. The chloride concentration rose after the administration of NaCl. The urea concentration fell to a lesser degree and hence there was only a small increase in total osmotic concentration.

The response of adult and infant rats to hypertonic solutions of urea

Adult rats responded to an increased concentration of urea in the body fluids in much the same way as human adults have been shown to do (McCance & Young, 1944), and the response was similar to that seen after the administration of hypertonic NaCl solution (Table 3). A very large diuresis was induced

TABLE 3. The response of adult rats which had been kept without water for 18 hr. to the administration of a hypertonic urea solution by stomach tube

Time	Minute volume c.c./1000 g. rat	Urine: Osmotic pressure m.osmol./l.	Urine: Chloride m.equiv./l.	Urine: Urea m.mol./l.	Blood urea m.mol./l.
10.0 a.m.–12.0 noon	0·003	3000	350	1240	6·6
12.30 p.m. 5% of the body weight 20% urea given					
12.30–1.0 p.m.	0·122	1360	125	750	—
1.0–2.0 p.m.	0·138	1410	105	1050	—
2.0–3.0 p.m.	0·138	1430	88	1060	—
2.30 p.m. Blood collected					40
3.0–4.6 p.m.	0·139	1460	62	1160	—
4.6–5.4 p.m.	0·111	1500	55	1175	—
5.4–6.0 p.m.	0·098	1580	38	1190	—
6.0–10.0 p.m.	0·017	2560	31	2160	—

by strong urea solutions given by stomach tube, and the urine produced during this diuresis was much more dilute than the preliminary urine. A difference from the response to NaCl solution lay in the fact that, in spite of the rising blood urea, there was a fall in the urea concentration of the urine as the diuresis developed. The chloride concentration fell and remained low. About seventy newborn rats were used for the urea experiments. The newborn rats responded to strong solutions of urea much as they did to those of hypertonic salt solutions (Table 4) and hence both these solutions demonstrate similar differences in behaviour between the kidneys of adults and of newborn animals. The minute volume was increased by a very small amount and the osmotic pressure rose slightly. The concentration of urea in the urine increased nearly

TABLE 4. The response of newborn rats to the administration of hypertonic urea solution by stomach tube

Time	Minute volume c.c./1000 g. rat	Urine: Osmotic pressure m.osmol/l.	Urine: Chloride m.equiv./l.	Urine: Urea m.mol./l.	Blood urea m.mol./l.
9.15 a.m.–12.15 p.m.	0·032	457	81	122	13
12.20 p.m. Blood collected and 5% of the body weight of 20% urea given					
1.0–5.30 p.m.	0·052	483	36	360	88
5.35 p.m. Blood collected					

three times, but the chloride concentration fell so that the total osmotic pressure of the urine did not rise very much. It is to be noted that there is very little variation in the volume or total concentration of the urine of the newborn animals in spite of the great increase in the urea in the blood.

Response of adult and newborn rats to water

The failure of hypertonic solutions of both salt and of urea to provoke a diuresis in newborn rats suggested an investigation of their response to administration of water. The adult response to water was the one to be expected from all previous work on water diuresis. Administration of water by mouth to adult rats caused a rapid rise in minute volume, while the osmotic pressure of the urine fell to a correspondingly low level. The concentrations of chloride and of urea in these urines became, naturally, very low. Experiments were performed on fully hydrated rats as well as on those that had been deprived of water for 18 hr. It was found that this short period of dehydration made no fundamental difference to the response of adult rats to 5% of their body weight of water. Both series of animals produced a large volume of much more dilute urine. The figures of a typical experiment are given in Table 5. When newborn rats were given a comparable amount of water their response was very different. It can be seen from Table 5 that the introduction of this

TABLE 5. Response of fully hydrated adult rats and of newborn rats to 5% of their body weight of water given by stomach tube

Time	Minute volume c.c./1000 g. rat	Osmotic pressure of urine m.osmol./l.	Concentration of urea in urine m.mol./l.
	Adult rats		
10.5 a.m.–12.30 p.m.	0·0086	2980	1230
12.30 p.m. Water given			
12.30–1.50 p.m.	0·071	414	174
1.50–2.45 p.m.	0·188	206	84
2.45–4.45 p.m.	0·031	1060	648
	Newborn rats		
10.10 a.m.–12.45 p.m.	0·024	510	375
12.45 p.m. Water given			
1.0–3.0 p.m.	0·025	489	333
3.0–5.0 p.m.	0·032	419	292

volume of water caused no rapid diuresis in these animals in the time taken by the adults to get rid of their excess of water. The minute volume increased only very gradually and the change in the osmotic pressure of the urine was correspondingly small. A comparison of the concentrations of urea in the urines of adult and of newborn animals emphasizes the fixed composition of the urines of these newborn rats.

It was clear from these experiments that there must be a fundamental difference in the response of adult and of newborn rats to water, and that at some stage in the young animal's development a change in function must take place. The young rats were not injured by these experiments and could be returned to the mother at the end of the day, so some of the litters were investigated again at 4, 8 and 12 days of age. Some of the results are given in Tables 6 and 7. It will be seen that by the time the animals were four days old

TABLE 6. Response of 4-day-old rats to water given by stomach tube

Time	Minute volume c.c./1000 g. rat	Urine: Osmotic pressure m.osmol./l.	Urine: Urea m.mol./l.
9.40 a.m.–12.40 p.m.	0·027	590	164
12.0 noon–12.20 p.m. 5% of the body weight of water given			
12.20–2.0 p.m.	0·107	310	132
2.0–3.30 p.m.	0·086	242	104
3.30–5.15 p.m.	0·029	565	190

TABLE 7. Response of 12-day-old rats to water given by stomach tube

Time	Minute volume c.c./1000 g. rat	Urine: Osmotic pressure m.osmol./l.	Urine: Urea m.mol./l.
10.0 a.m.–12 noon	0·049	600	230
12.0 noon 5% of the body weight of water given			
12.0 noon-1.0 p.m.	0·138	452	99
1.0–2.0 p.m.	0·197	188	33
2.0–4.25 p.m.	0·051	520	177

they were beginning to respond to the dose of water in adult fashion by a small diuresis and by a small fall in the osmotic pressure of the urine. At 4 days old, however, the osmotic pressures show that rats still produce a dilute urine and are probably unable to concentrate it as much as an adult can. At 12 days of age the response to water was almost indistinguishable from that of the adult animal but the urine was still dilute in the preliminary sample and at the end of the experiment it returned to this state. This consistently dilute urine is typical of suckling animals.

DISCUSSION

If the function of the kidney is considered to be primarily the regulation of the internal environment, then the introduction of water or hypertonic solutions into the body automatically subjects the organ to a task which it may or may not be able to fulfil. In the experiments in which water was administered, the kidneys of the adult animals proved themselves quite able to restore, if not absolutely to maintain, the constancy of this internal milieu. This was expected. The kidneys at birth, however, were not able to do so. When water alone was administered to newborn rats little was excreted and the tissue fluids must have remained correspondingly diluted. This was, however, evidently not a severe physiological handicap to the animals for they appeared little the worse, and were usually accepted by their mothers at the end of the experiment and reared successfully. By 4 days of age the animals were much better served by their kidneys and excreted 20 % of the test dose in the first hour and altogether 35 % in 5 hr. Adult animals excreted from 40 to 60 % of the test dose in 5 hr.

To administer hypertonic solutions to animals which are already hydropenic, subjects them to a severe physiological strain. If the solution introduced is more hypertonic than the most concentrated urine which the kidney can produce, the kidney can only excrete the administered crystalloid by drawing upon some of the body water of which there is already a shortage. This is the situation which arises if a thirsty man drinks sea water. It is of benefit to the animal, however, that the kidney should draw upon the body water in this way, for in adult life the kidney can always excrete a much more concentrated solution than the animal could endure in its internal environment. In the urea experiments quoted in this paper the adults excreted about 27 % of the administered urea in the 5 hr. of observation; the newborn animals only 6 %. In the salt experiments the adults excreted 26 % of the dose; the newborn animals 5 %. Thus in both experiments the kidneys of the newborn animals showed themselves much less capable of dealing with these changed conditions. It is not a new observation that the kidneys of newborn animals are not such effective organs as they will become in adult life. It has been shown by Adolph (1943) that young dogs excrete water less rapidly than adult animals and the same is probably true of young children (Lasch, 1923), but these observations need confirmation. The kidneys of newborn rats, however, seem to be considerably less effective than those of newborn dogs or infants for they excrete no more than traces of the test dose. The sodium chloride and the urea clearances of human infants are known to be well below adult standards when the two are compared on the basis of surface area or body weight (McCance & Young, 1941; Gordon, Harrison & McNamara, 1942). These clearances have not been compared at similar periods of life in other animals but it is evident that such differences also exist in rats. Other peculiarities of renal function in the early days of life have been discussed by McCance (1946). These aspects of developmental physiology must be of some significance to anyone interested in the development and treatment of young children, but it may be some little time before they are fully explained.

SUMMARY

1. When 10 % sodium chloride solutions were administered by mouth to adult rats the animals responded by reducing the osmotic pressure of their urine and by producing a large diuresis which enabled them to excrete 27 % of the crystalloid in 5 hr. When a similar dose was given to newborn rats they responded by raising slightly the osmotic pressure of the urine, but they did not produce a diuresis and they excreted only 6 % of the test dose in 5 hr.

2. Hypertonic solutions of urea produced effects similar to those of NaCl in both the adult and the newborn rats.

3. When 5% of the body weight of water was administered to adult rats the diuresis was rapid and effective but newborn rats did not respond to similar treatment in the same way. They produced no significant diuresis and no dilution of the urine.

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