

Acta, 1962, v63, 403.

9. Brooks, J. B., Lewis, V. J., Pittman, B., Proc. Soc. Exp. Biol. and Med., 1965, v119, 748.

10. Dreesman, G., Larson, C., Pinckard, R. N., Groyon, R. M., Benedict, A. A., *ibid.*, 1965, v118,

292.

11. Porter, R. J., *ibid.*, 1966, v121, 107.

12. Finkelstein, M. S., Uhr, J. W., Science, 1964, v146, 67.

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Diuresis in Newborn Rat Given Intravenous Water or Salt Solution.* (31134)

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Diuresis in response to intragastric administration of water or salt fails to occur in newborn rats(1), dogs, or humans(2). Absence of diuresis has been attributed to the state of certain renal structures and to non-functioning of the antidiuretic hormone system or its renal effects. The present work aimed to explore factors of manipulation that might influence diuresis. Route of administration proved to be one factor, for intravenous fluid aroused diuresis.

Procedure. Rats were mostly used at 3-26 hours after birth. Each was taped to a board and kept in an incubator after preparation. Fluids were injected into the femoral vein (I.V.) through a cannula finely drawn from No. 10 polyethylene tubing and inserted during temporary ether anesthesia. A urethral catheter (No. 10 tubing) was inserted into the urinary bladder, outside a thin glass probe which was then withdrawn. The catheter led the urine into a calibrated glass capillary to measure its flow. Fluid was injected 0.5 to 1 hour after preparation was completed. Chloride analyses of 4 μ l volumes of urine or plasma employed a microburette and the method of Natelson(3). In other tests, fluids were injected into the peritoneal cavity (I.P.). Radioactive Na²² was sometimes added to these fluids so that movement of injected Na could be traced.

Results. After I.P. injection of water, only

a small diuresis was discerned; urine flow in the second half hour was significantly greater ($P = .02$) than the mean of all control periods. This urine flow did not differ from that following loading by stomach tube (S.T.) (reported in(1)). Diuresis was almost as small when 1 M NaCl instead of distilled water had been given (Table I). Rats 3 days of age similarly treated produced little more urine in 1.5 hours after water had been given ($1.3 \pm .14\%$ b.w.), (b.w. = body weight), and no more urine after 1 M NaCl had been given. Peritoneal fluid equalled the plasma in chloride concentration at 0.6 hour. During that period of time the intraperitoneal volume, as collected in sacrificed animals, had not diminished. Na²² recovery then showed 1% of the dose in urine, and only 6% in peritoneal fluid. Cl in peritoneal fluid equalled 9% of the amount injected. Plasma chloride concentration remained greatly elevated for at least 18 hours.

After I.V. injection, completed in 6 minutes, diuresis began about 4 minutes later (Fig. 1). The small delay was the same whether the injecta were hypotonic or hypertonic to the blood plasma.

Administration I.V. of 1 M NaCl resulted in (a) the full return of the volume of the injecta (4% of b.w.) in the urine of the newborn within 1.5 hours, and (b) even greater urinary volume at 2-5 days of age, 5.2% b.w. (Fig. 1, B). Urine production after I.V. loading was 4-fold greater when 1 M NaCl was given than when water was given.

Maximal concentrations of chloride in urine

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TABLE I. Urine and Plasma After Fluid Administrations to Newborn Rats, ± Standard Errors.

	Time (hr)	Urine						Plasma	
		Cum. flow, % B.W.			Cl conc, meq/l			I.V.	I.P.
		I.V.	I.P.	I.V.	I.P.	I.V.	I.P.		
4% B.W. Distilled water	0	*.15 ± .06	*.07 ± .02	66 ± 3	46 ± 10	.025 ± .005	.010 ± .004	101 ± 0.6	104 ± 1
	0.5	.46 ± .07	.04 ± .01	54 ± 6	†53 ± 14	.024 ± .005	†.003 ± .001		
I.V. I.P.	1.0	.92 ± .10	.30 ± .04	37 ± 7	30 ± 5	.041 ± .010	.010 ± .002		
N = 6	1.5	1.08 ± .22	.32 ± .07	33 ± 10	18 ± 2	.046 ± .010	.014 ± .002		
	2.0	1.17 ± .23	.65 ± .09	41 ± 11	21 ± 4	.050 ± .011	.017 ± .003	98 ± 1.3	98 ± 1
4% B.W. 1 M NaCl (4 meq/100 g)	0	*.23 ± .44	*.14 ± .02	87 ± 5	88 ± 8	.045 ± .017	.070 ± .01	103 ± 0.9	99 ± 2
	0.5	2.07 ± .39	.13 ± .05	165 ± 3	†150 ± 22	.335 ± .055	†.047 ± .03		153 ± 2
I.V. I.P.	1.0	3.72 ± .24	.45 ± .14	172 ± 5	181 ± 9	.618 ± .045	.083 ± .03		
N = 8	1.5	4.13 ± .28	.61 ± .20	197 ± 7	223 ± 6	.698 ± .075	.121 ± .04		
	2.0	4.38 ± .39		204 ± 10		.755 ± .082		152 ± 2.1	158 ± 2
2% B.W. 1 M NaCl (2 meq/100 g)	0	*.10 ± .04		77 ± 19		.019 ± .054		102 ± 0.8	
	0.5	.90 ± .12		154 ± 9		.137 ± .030			
I.V.	1.0	1.56 ± .16		176 ± 8		.232 ± .037			
N = 5	1.5	1.77 ± .23		194 ± 9		.256 ± .038			
	2.0	1.91 ± .27		181 ± 7		.274 ± .035		128 ± 2.6	
4% B.W. 0.1 M NaCl I.V. (0.4 meq/100 g)	0	*.12 ± .04	*.36 ± .08	Suckled	Not suckled	Suckled	Not suckled	Suckled	Not suckled
	0.5	.40 ± .13	1.54 ± .21	95 ± 18	110 ± 5	.033 ± .011	.115 ± .031	101 ± 1.6	105 ± 1.1
Suckled	1.0	.60 ± .16	2.10 ± .25	74 ± 15	98 ± 4	.035 ± .016	.185 ± .023		
Not suckled	1.5	.75 ± .23	2.47 ± .30	77 ± 23	98 ± 3	.049 ± .020	.241 ± .020		
N = 5	2.0	.85 ± .26	2.84 ± .37	65 ± 13	107 ± 5	.062 ± .026	.282 ± .019		
				68 ± 7	109 ± 9	.070 ± .029	.321 ± .021	104 ± 3.0	108 ± 1.3

* 0 = % B.W./0.5 hr preceding injection

† N = 4

‡ N = 3

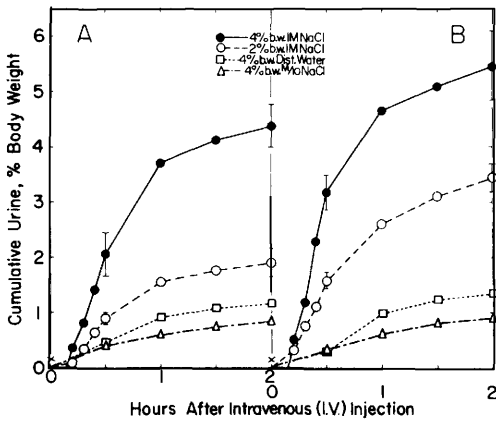


FIG. 1. Cumulative production of urine following I.V. injections into neonates less than 1 day old (A), and into infants 2-5 days old (B). Mean urine flow during $\frac{1}{2}$ hour preceding all injections is also shown (X). All ratlets suckled.

or in plasma were alike, whether salt was loaded I.P. or I.V. Although total chloride output totalled 6-fold more when loaded I.V. than I.P., the load of chloride remaining in the body at 2 hours was still 3.2 meq/100 g b.w., or 80% of that given. Injection I.V. of 2% b.w. of 1 M NaCl instead of 4% b.w. made no difference in concentration of chloride attained in the urine, but plasma chloride concentration increased only half as much.

Injection of distilled water I.V. resulted in return of only one-fourth the given volume. But such diuresis in newborn individuals was more than double the small diuresis after I.P. loading. No hemoglobin appeared in the plasma or in the urine after I.V. water.

A second controllable factor in diuresis was as follows. Rats interrupted during the first suckling after birth produced 3 times as much urine as suckled rats of the same age, each injected I.V. with 0.1 M NaCl. Rats separated from the dam immediately after birth (non-suckled) regularly exhibited this larger diuresis as compared with suckled rats of the same litter and age (Table I).

Discussion. Even on the day of birth, if fluids were given I.V. (but not if given I.P.), (a) the rat distinguished between loads of hypertonic solutions and loads of hypotonic solutions, or water and (b) factors controlled by the experimenter played decisive roles in excretion.

Absence of diuresis in response to fluid placed in the newborn stomach was thought to be related to known age differences in circumnatal kidney structure and function(4,5). However, suitable arousal now shows the functions to be adequate for modest diuresis. Again, absence of antidiuretic hormone in assays of newborn blood and lack of response to this hormone were considered to deny the newborn any means of arousal of diuresis(5). Apparently the hormone, even when present, does not suppress diuresis when water is given S.T. or I.P. within 3 days of birth(1,2).

Possibly expansion of the vascular bed accounts for part of the diuresis after I.V. injection of fluid. The increase of blood volume was presumably greater (than if given I.P.) at the end of injection of water or dilute saline. The ensuing diuresis was delayed (10 minutes) as much after dilution as after strong salt was added which probably by osmosis enlarged the volume further. The amount of the salt diuretic response was proportional to the amount of chloride injected. The salt may have maintained hypervolemia longer.

Increase of intravascular pressures, perhaps within the kidneys, may contribute to diuresis in the newborn rat. Other agents that induce diuresis of the newborn rat (hypoxia, epinephrine)(6), characteristically act upon blood vessels. Hypoxic diuresis appears only after delay, as do the salt and water diureses.

Minute size of the diuresis after S.T. administration of fluid to newborn is partly accounted for by slow absorption of water and of salt(1,7). Minute size of diuresis after I.P. administration is more difficult to understand, since I.P. fluids, collected by incision and drainage at sacrifice, show that both sodium and chloride given in 1 M concentration mostly leave the peritoneal cavity within 0.6 hour, even as in adult rats(8,9).

In the newborn rat, 60% of the total body fluid, or 52% of the fat-free body(10), resembles the plasma in chloride concentration. Approximately this volume receives the salt administered as 0.1 M NaCl. A still larger volume of distribution becomes available through osmosis when the salt is administered as 1.0 M NaCl.

TABLE II. Body Fluid Loads* 2 Hours After 4% B.W. of 1 M NaCl.

Postnatal age	Route of administration		
	S.T.	I.P.	I.V.
Newborn (3-26 hr)	+3.4†	+3.4†	— .4
Infant (2-7 days)	+1.7	+3.4†	-1.5
Adult	+ .9	-1.1	

* Load = injecta-urine, in % B.W.

† Experiment completed at 1.5 hr.

‡ 2 days of age was youngest tested S.T.

After 1.0 M NaCl was placed I.P., the solute which escaped within 0.6 hour from the peritoneal cavity undoubtedly escaped *via* the blood stream. Its distribution was thereafter similar to that when NaCl was placed I.V., except for the small fraction, 6 to 9% of the dose, left in the peritoneal cavity if originally put there. Hence, known differences of solute distribution do not account for differences in diuresis or in chloruresis resulting from the two sites of injection.

Before the newborn rat first suckles, it has a special capacity to excrete excess fluid in the urine. Suckling might have decreased this capacity through (a) nutritional influences from the first ingesta, or (b) alimentary tract alterations at that time. Possibly more important is the fact that the non-suckled rat is relatively rich in chloride, as indicated by its higher initial plasma chloride ($P = .05$) which persisted during the diuresis. The greater chloruresis in the non-suckled rats indicates that they readily release added chloride and rapidly move added fluid from the blood stream.

Overall fluid retentions (Table II) show that the I.V. loaded newborn released barely any body fluid in support of diuresis after NaCl, whereas the older infant sustained a deficit of 1.5% b.w. The adult rat given 1 M NaCl I.P. suffered a body fluid deficit of 1.1% b.w.(9), increasing to 4.2% b.w. at the end of 4 hours, at which time the plasma chloride concentration was elevated by only 23 meq/l. In response to I.V. injections of 1 M NaCl, the older infant rat, but not the newborn, partly imitated the adult response to I.P. injections by producing urine at the sacrifice of body fluids. At 1.5 hours after I.P. injection into newborns, there was no

sacrifice of body fluid; instead the body retained 3.4% b.w. out of 4.0% b.w. administered.

Diuresis in the newborn lasted only one hour, while in the adult it lasted until most of the salt load or the water load had been excreted. This fact indicates that the newborn has the machinery for diuresis, but is not stimulated to use it for long. At best the newborn eliminated only 20% of the chloride given.

Possibly the retention of body chloride and water which characterizes the fetus has to change, especially within extracellular compartments, before post-natal acquisition of (a) sustained diuresis, (b) higher urinary chloride concentration, and (c) regulation of total body chloride.

Summary. 1. The newborn rat shows diuresis when given 1 M NaCl solution by vein. The urine output in 2 hours is 6-fold that after administration of either intraperitoneal or intragastric fluid. 2. Water by vein induces twice as much diuresis as does water by other routes. 3. Plasma and urine chloride concentrations change equally, regardless of route of fluid administration; only time relations differ with route. 4. When newborn rats are not allowed to suckle, saline diuresis is 3-fold that in suckled litter-mates. 5. Controllable factors therefore determine whether water or salt shall arouse diuresis on the day of birth.

1. Falk, G., *Am. J. Physiol.*, 1955, v181, 157.
2. Ames, R. G., *Pediatrics*, 1953, v12, 272.
3. Natelson, S., *Microtechniques of clinical chemistry for the routine laboratory*, C. C Thomas, Springfield, Ill., 1957, p165.
4. Boss, J. M. N., Dlouha, H., Kraus, M., Krecek, J., *J. Physiol.*, 1963, v168, 196.
5. Krecek, J., Heller, J., *Proc. XXII Internat. Congress Physiol.*, 1962, v1, 53.
6. Hoy, P. A., Adolph, E. F., *Am. J. Physiol.*, 1956, v187, 32.
7. Heller, J., *Physiol. Bohemoslov.*, 1963, v12, 526.
8. Adolph, E. F., Northrop, J. P., *Am. J. Physiol.*, 1952, v168, 311.
9. Adolph, E. F., Barker, J. P., Hoy, P. A., *ibid.*, 1954, v178, 538.
10. Jelinek, J., *Physiol. Bohemoslov.*, 1961, v10, 259.

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