THE RESPONSE OF NEWBORN RATS TO ADMINISTRATION OF WATER BY THE STOMACH

By H. HELLER

From the Department of Pharmacology, University of Bristol

The antidiuretic hormone content of newborn rat pituitary glands has been shown to be much lower than that of the glands of adult animals (Heller, 1947). This finding, when correlated with morphological evidence for the immaturity of the mammalian neurohypophysis at birth, suggested the possibility of neurohypophysial hypofunction in relation to the kidney of the newborn. If such hypofunction existed, the water metabolism of the newborn would be expected to show certain features which have been observed in cases of diabetes insipidus in the adult. For instance, Klisiecki, Pickford, Rothschild & Verney (1933) observed an earlier onset of diuresis after water administration by mouth when the responses of a normal man were compared with those of a man suffering from diabetes insipidus. The response of newborn rats to water administration was therefore investigated.

METHODS

Experimental animals. 547 male albino rats, 18–42 hr. old (weighing from 4·2 to 6·5 g.) and ninety-three male adult rats were used. The adults, which were of the same strain as the newborn animals, were kept on a standard diet.

The mode of administration of water to newborn rats. Attempts to give the standard dose of water, 4·5 c.c./100 g. rat, by mouth were rarely successful. Intra-abdominal or intramuscular injection led to considerable and protracted losses from the site of the injection. The following method of water administration was finally adopted: the animals were removed from the breeding cages shortly after they had been suckled, i.e. when milk in the stomach could be seen through the abdominal wall. Water was then injected into the stomach through the abdominal wall, the milk content of the stomach serving as a guide for the injection needle. Water warmed to about 35° C. had to be administered to avoid sudden gastric contractions.

A series of control experiments were done to ascertain that water administered in this manner was quantitatively placed into the alimentary tract. A number of newborn rats were weighed on an analytical balance, water injections made as above, and the animals weighed again. The precise amount of water given could thus be determined. It amounted to $4·54 + 0·041$ (s.e.) g./100 g. in a series of twelve animals. The rats were then killed by decapitation, cardia and rectum ligatured and the gastro-intestinal tract weighed. The mean difference between the weight of the alimentary

tract of these animals and that of twelve litter controls was 4.58 ± 0.454 g./100 g. showing that the stomach and the intestine contained all the injected water up to 6 min. after the intragastric injection.

The collection of urine in newborn rats. The rats were placed singly into small beakers lined with filter paper. Animals which passed urine during the period of experimentation were discarded. In other series, quantitative collection of urine was ensured by ligature of the penis. Statistical comparison of a number of series showed that the urinary output in the latter animals was the same as in newborn rats whose penis had not been tied ($P > 0.9$).

Control experiments showed that it is not always possible to empty the bladder of newborn rats by suprapubic pressure. To collect the bladder urine quantitatively the animals were, therefore, killed by decapitation and the urine withdrawn by bladder puncture. The quantities thus obtained were so small (ranging approximately from 0.01 to 0.15 g. of urine) that it seemed preferable to weigh the urine rather than to rely on determinations of volume. Moreover, the specific gravity of the urines of newborn rats, collected under various experimental conditions, varied so little that it seemed permissible to equate weight with volume without undue loss of accuracy.

Determinations of extrarenal water loss

Newborn rats. The extrarenal loss was determined by weighing the rats on an analytical balance. Animals which passed faeces or urine were discarded. In later series the penis was tied to avoid loss of urine. Comparison showed that the rate of extrarenal loss was not changed by this procedure ($P > 0.9$).

Adult rats. The animals were put singly into glass metabolism cages fitted with urine/faeces separators. The body weight, the weight of the urine and that of the faeces were determined from hour to hour. The urine was collected under paraffin oil. The air temperature in these as well as in the experiments on newborn rats was controlled thermostatically. No attempt, however, was made to control air humidity.

The choice of air temperature at which experiments on newborn rats were performed

It is not easy to decide at which environmental temperature experiments on newborn rats should be conducted. Full capacity for thermoregulation is only acquired by rats during the fourth week of extra-uterine life (Brody, 1943). For instance, Gullick (1937) showed that rats aged 6 days kept at air temperature ranging from 24 to 37° C. acquired a rectal temperature approximately 1·5° C. higher than that of the surrounding air while the rectal temperature dropped to less than 0·5° C. above that of the air if such animals were exposed to temperatures of 20° C. or lower for 1 hr. Newborn rats derive much of their warmth from the mother, i.e. from the contact with a surface at about 31° C. (Herrington, 1940), and this would suggest that 'physiological' experiments on newborn rats should be performed at about this temperature. However, it should be considered that the mother leaves the young rats for considerable periods (e.g. when feeding) with the result that lower body temperatures are likely to occur in newborn rats. These physiological fluctuations of body temperature can hardly be imitated experimentally and it was therefore decided to conduct experiments at two levels of air temperature. The levels chosen were: (1) the average room temperature (20/21° C.), and (2) that of the approximate skin temperature of adult rats (30/31° C.). The experimental results on newborn rats were therefore obtained at the lowest and at the highest temperatures which these animals were likely to encounter.

RESULTS

The effect of water administration on the urine flow of newborn rats

The conduction of diuresis experiments on normal adult rats is much facilitated by the ease with which the animals can be made to void urine at any time chosen by the investigator: prodding and, if necessary, suprapubic abdominal

pressure is sure to induce complete emptying of the bladder. In newborn rats, gentle stroking of the perineal region, aided by digital pressure on the abdomen, frequently induced voiding of considerable volumes of urine, but control experiments showed that this procedure led to complete emptying of the bladder in a minority of newborn animals only. A series of twenty-five rats, weighing from 4·85 to 6·14 g., were made to deliver as much urine as possible by suprapubic pressure, the animals were then decapitated, and residual urine withdrawn by direct puncture of the bladder. The figures for the mean weight of residual urine of the series were 0·0293 ± 0·00532 (s.e.) g. or 0·52 ± 0·091 g./100 g. In view of these results the following procedure was adopted to show the rate of water diuresis in newborn rats. The bladder of a number of male litter mates was 'emptied' by suprapubic pressure. The animals were then divided into two series. One series received about 4·5 c.c. water/100 g. rat by injection into the stomach; the second series received no water. After a given time the animals in both series were killed and the urine withdrawn by bladder puncture. The urines were weighed singly in tared weighing bottles. A significant difference between the mean urinary weights of the two series would thus be due to the renal excretion of the administered water. However, it will be seen from Fig. 1 A, which shows the results of experiments conducted at 30/31° C., that there was no significant difference between the injected and the control series even when the period allowed for renal excretion of the injected water was extended to 5 hr. The mean weight of the urine in a series of eleven newborn rats killed 1 hr. after water administration was 0·96 ± 0·073 (s.e.) g./100 g., that of the control series (eleven animals) was 1·01 ± 0·092 g./100 g., the figures for animals killed after 2 hr. were 1·12 ± 0·151 g./100 g. (twelve injected animals) and 1·17 ± 0·142 g./100 g. (eleven controls), those for animals killed after 3 hr. were 1·68 ± 0·115 g./100 g. (twenty-two injected animals) and 1·81 ± 0·088 g./ 100 g. (twenty-three controls) and those for rats killed after 5 hr. 2·16 ± 0·114 g./ 100 g. (fourteen injected animals) and 2·13 ± 0·114 g./100 g. (fourteen controls). It should be pointed out that the figures for weight of urine given have to be corrected by deduction of the 'residual urine' (0·52 g./100 g. rat) to represent the urinary output during the periods stated (Fig. 1 A).

No evidence could be obtained from these experiments for the occurrence of water diuresis in newborn rats. Experiments done at 20/21° C. air temperature showed further that the absence of water diuresis was not due to 'heat inhibition' (Heller & Smirk, 1932*b*; Weiner, 1944) at the higher temperature level: the mean amount of urine produced by twenty-one newborn rats injected with water was 1·03 ± 0·078 g./100 g. after 4 hr. at 20/21° C.; that of the control series (twenty-two animals) was 1·04 ± 0·122 g./100 g.

Fig. 1 B shows the renal response of adult rats which received the same relative amount of water (4·5 c.c./100 g. rat). Control experiments had shown

that the gastro-intestinal tract of the newborn rats contained approximately 3 % of their body weight of milk at the outset of the diuresis experiments ($2\cdot71 \pm 0\cdot318$ g./100 g. rat in a series of twenty animals). The experiments on adult rats were therefore conducted as follows: the animals were starved for 24 hr. but received water *ad libitum*; 3 c.c. cows' milk/100 g. rat was then given by mouth and 4·5 c.c. water/100 g. 10 min. later. Fig. 1 B shows that water diuresis proceeded freely in these animals.
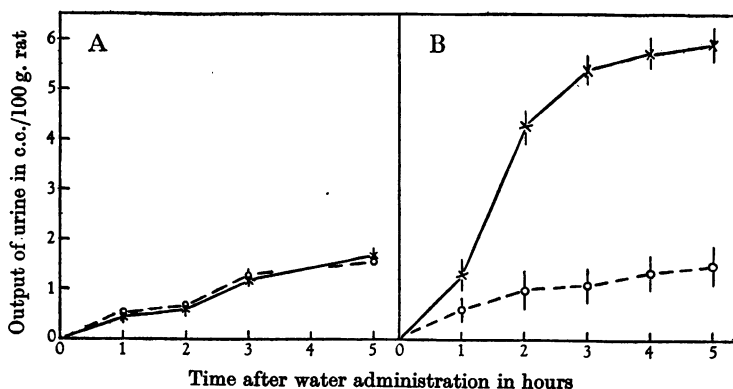

Fig. 1. Comparison between the urinary output of newborn and of adult rats after administration of water by the stomach.

A.　×——× =mean urinary output of newborn rats after the intragastric injection of 4·5 c.c. water/100 g. rat. ○－－－○ =controls. The figures for the urine volume at various times were obtained by deducting the mean residual urine volume (=mean volume of urine contained in the bladder at the outset of the experiment) from the volume of urine obtained at a given time.

B.　×——× =mean urinary output of adult rats which received 3 c.c. milk/100 g. by mouth and 4·5 c.c. water/100 g. 10 min. later (3 c.c. milk/100 g. rat =approximate content of milk in alimentary tract of newborn rats at outset of diuresis experiments). ○－－－○ =mean urinary output of adult rats which received 3 c.c. milk/100 g. only. The vertical lines indicate the standard error. There is no evidence that the newborn rats excreted any of the administered water during the 5 hr. of observation.

Further diuresis experiments on adult rats receiving a preparation of 'full cream dried cows' milk,' partly reconstituted to make the concentrations of proteins and fat approximately equal to those of rat milk, gave essentially the same results.

The rate of gastro-intestinal absorption of water in newborn rats

Little seems to be known about the rate of alimentary water absorption in newborn mammals. This factor was, therefore, investigated. The method used to determine the rate of gastro-intestinal water absorption was essentially the same as that used by Heller & Smirk (1932 a) in adult rats. The weights of the alimentary tracts of series of rats, killed at given times after the administration of a standard dose of water by stomach tube, were compared with the mean gastro-intestinal weights of a control series. However, the technique of intragastric injection of water into newborn rats made it necessary to modify this simple procedure. It will be remembered that, for intragastric injections, animals had to be used whose stomachs were not empty. A decrease in the weight of the alimentary tract of the injected animals after a given time would therefore

be due not only to the absorption of the injected water but also to the absorption of an unknown quantity of milk. To overcome this difficulty the experiments on newborn rats were conducted as follows. A number of newborn animals (litter mates) were weighed and divided into two series. The first animal was injected with the standard dose of water and the time of injection noted. An animal of the other series was then set aside as a control. The injected animal was weighed to determine the extra water load accurately. This procedure was followed until each of the series of injected rats was provided with a control. After a given time the animals were killed and their alimentary tracts weighed on an analytical balance. The difference between the mean weight of the alimentary tracts of the injected rats (in terms of g./100 g. rat) and that of the
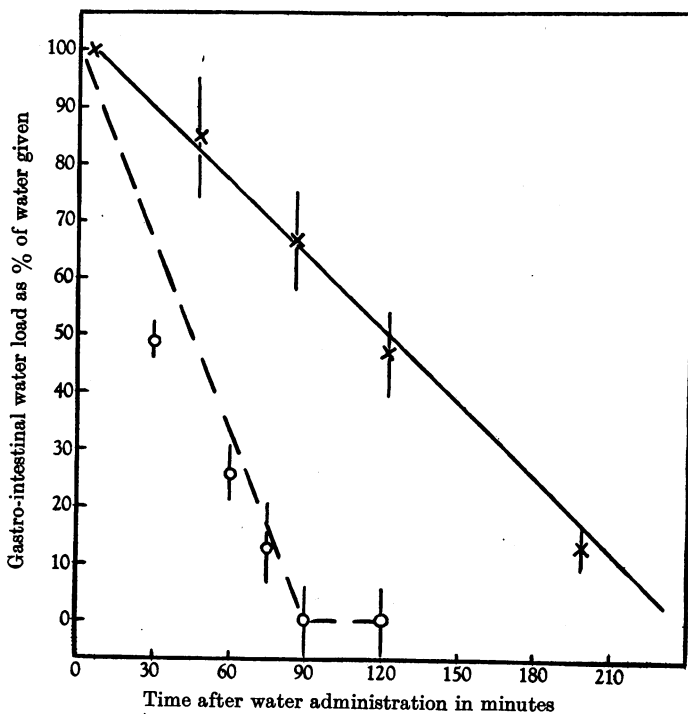

Fig. 2. The rate of alimentary water absorption in newborn (×——×) and in adult (◯- - -◯) rats. Both series received 4·5 c.c. water/100 g., the adult rats, in addition, received 3 c.c. milk/ 100 g. 10 min. before the water was administered. The newborn rats were kept at 30/31° C. air temperature, the adult rats at 20/21° C. For results on newborn rats kept at 20/21° C., see text. The slower rate of water absorption in newborn rats is evident.

alimentary tracts of the control series indicated the fraction of the administered water not absorbed. Estimations of urinary weight were not required in these series and the penis was therefore not ligatured. However, two additional series of experiments showed that the rate of intestinal water absorption in newborn rats was not appreciably changed by this procedure.

Experiments on adult rats were conducted in the same manner as in newborn rats except that food (but not water) was withheld for 24 hr. and that the animals received 3 c.c. milk/100 g. 10 min. before 4·5 c.c. water/100 g. was administered.

Fig. 2 shows clearly that the newborn rats absorbed water at a considerably lower rate than adult animals. For instance, 90 min. after the administration

of water, when absorption in the adults was complete, more than 60 % of the water given was still in the alimentary tract of a series of newborn animals kept at 30/31° C. The rate of gastro-intestinal water absorption in newborn rats was also studied in animals kept at 20/21° C. A statistical comparison of the figures for the mean alimentary water load 48 and 90 min. after water had been given with those obtained at the corresponding times in animals kept at 30/31° C., showed that the rate of water absorption was approximately the same at both temperatures (t after 48 min. $= 0.263$, $P > 0.7$; t after 90 min. $= 0.261$, $P > 0.7$) indicating that the body temperature, and therefore the metabolic rate, had little or no influence on the gastro-intestinal absorption of water.

The rate of extrarenal water loss of newborn rats

It is known (Heller & Smirk, 1932a) that water diuresis in the adult rat, and in adults of other mammalian species (Klisiecki *et al.* 1933), begins only when intestinal water absorption is almost complete. The extrarenal water loss during this period of latency diminishes the volume of extra water available for renal excretion but the short duration of the latency period in adult rats makes this decrease comparatively negligible (Heller & Smirk, 1932a). It seemed likely that these relationships would not be the same in newborn rats. First, it had been shown that alimentary water absorption proceeded at a much lower rate than in adults (the absorption of the standard dose of water taking 180–210 instead of approximately 90 min.) and secondly that no sizable part of the extra water load was excreted by the kidneys up to 5 hr. after administration. The question arose therefore whether the extrarenal water loss during the long period of alimentary absorption was of such magnitude that it accounted for the absence of water diuresis in newborn rats. In other words, did the rate of extrarenal water loss in the newborn animals keep pace with the rate of alimentary water absorption with the result that the extra-alimentary water load became negligible or, at any rate, insufficient to sustain a water diuresis?

Determinations of the rate of extrarenal water loss of newborn and of adult rats at two levels of air temperature gave the following results (means with their standard errors):

Extrarenal water loss:

Adult rats at 20/21° C. $= 0.448 \pm 0.0334$ (46) g./100 g./hr.
       at 30/31° C. $= 0.459 \pm 0.0327$ (46) g./100 g./hr.
Newborn rats at 20/21° C. $= 0.072 \pm 0.0011$ (43) g./100 g./hr.
       at 30/31° C. $= 0.380 \pm 0.0213$ (42) g./100 g./hr.

It is clear from these results, and from the data shown in Fig. 3, that, at the lower temperature level, the extrarenal water loss of the newborn animals was considerably lower than that of the adults. The extrarenal water loss of

newborn rats increased very significantly when the temperature was raised to 30/31° C., but this increased rate of water loss did not exceed that of adult animals at 20/21° C., i.e. at room temperature; in fact statistical analysis showed that the two rates were of the same order of magnitude ($t = 1 \cdot 67$, $P > 0 \cdot 09$). The extrarenal water loss of newborn rats kept at 30/31° C. was, if anything, lower than that of adult rats kept at approximately the same temperature ($t = 2 \cdot 02$, $P < 0 \cdot 05$).
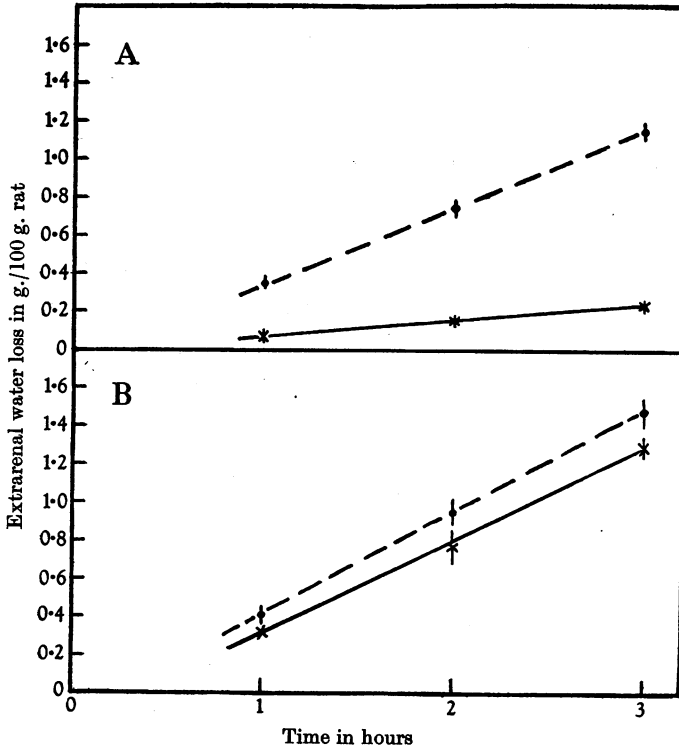

Fig. 3. Extrarenal water loss of newborn (A) and of adult (B) rats at two levels of air temperature. ×——× = extrarenal water loss at 20/21° C. ●---● = extrarenal water loss at 30/31° C. Results fully explained in text.

The striking difference between the rates of extrarenal water loss of newborn rats at different levels of air temperature is in agreement with the observations of Gullick (1937) and Brody (1943) who showed that rats during the first four weeks of extra-uterine life are not completely homoiothermal. These authors found that, in such animals, body temperature and basal metabolism were closely correlated with the air temperature.

It is evident from the reported data that the *rate* of extrarenal water loss of newborn rats was not higher than that of adults. However, it still remains to be shown how the protracted course of the alimentary water absorption

influences the tissue water load. In other words, given a rate of extrarenal water loss equal or lower than that of adults, what is the tissue water load of newborn rats at the time when the gastro-intestinal absorption of the administered water has been completed?

The following experiments were made to estimate this quantity which will be called the 'residual water load'. The bladder of a number of newborn rats was emptied, the penis ligatured and the body weight determined. Water was then administered by intragastric injection and the exact amount given (=initial water load) ascertained by reweighing. After a given time the rats were weighed again to estimate the extrarenal water loss, killed immediately after the last weighing, the bladder urine withdrawn and its weight determined. An equal number of non-injected controls were treated in an identical manner. It will be seen from Table 1 that there was no significant difference

TABLE 1. Estimation of 'residual' (=tissue) water load in newborn rats
after administration of water by the stomach

Duration of experiment (=hr. after water administration)	Air temp. in ° C.	No. of rats used	Amount of water administered (=initial water load) in g./100 g. rat	Extrarenal water loss during experiment in g./100 g.	Weight of bladder urine at end of experiment in g./100 g. (in brackets): weight of urine of control series (residual urine not deducted)	Difference between weight of urine in animals with extra water and controls (Fisher's t test)	Residual water load in % of initial water load
3	30/31	22	4.50 ± 0.080	1.43 ± 0.080	1.68 ± 0.115 (1.81 ± 0.088)	$t = 0.09$ $P > 0.9$	67.4 ± 1.86
5	30/31	19	4.70 ± 0.105	1.61 ± 0.106	2.16 ± 0.114 (2.13 ± 0.114)	$t = 0.01$ $P > 0.9$	62.8 ± 2.31
4	20/21	21	4.47 ± 0.083	0.41 ± 0.037	1.03 ± 0.078 (1.01 ± 0.122)	$t = 0.07$ $P > 0.9$	90.9 ± 0.86

Since gastro-intestinal absorption of the dose of water given (4.5 c.c./100 g. rat) was completed after about 3 hr. (see Fig. 2) and since the newborn rats did not excrete the extra water by the renal route (see the present table), the tissue water load at any time after completion of gastro-intestinal water absorption was equal to the 'initial water load' minus the extrarenal water loss. Results are given as means with their standard errors.

between the mean urine volumes in any of the three series of experiments performed. That is to say, none of the administered water had been excreted by the kidneys. The residual water load could therefore be calculated by deducting the extrarenal water loss from the initial water load. Table 1 shows that the mean residual water load in a series of rats kept at 30/31° C. was still about 70 % of the initial water load 3 hr. after water had been administered, i.e. at a time when alimentary water absorption had been completed. It is thus clear that it was not the extrarenal water loss which prevented a water diuresis in the newborn rats. Moreover, the high figures for the residual water load after 4 hr. at 20/21° C. and after 5 hr. at 30/31° C. showed (Table 1) that newborn rats were unable to excrete 'extra water' by the kidney even when comparatively large amounts of it were demonstrably lodged in the extra-alimentary tissues.

DISCUSSION

Evidence is accumulating that the water metabolism of newborn infants and animals differs in many points from that of the adults of the species. This could probably be expected from the very different cell milieu of newborn mammals but recent work indicates that these differences extend to the function of the kidney (McCance & Young, 1941; Heller, 1944) and that of integrative organs like the posterior pituitary gland (Heller, 1947).

The present investigation on the water metabolism of newborn rats is less concerned with any single functional factor than with the complex phenomenon of 'water diuresis'. The results show clearly that water diuresis, as found in adult animals, does not occur in newborn rats. No evidence could be obtained that a dose of water, placed in the alimentary tract of normally hydrated newborn rats, increased the urinary volume during 5 hr. after water administration. Experiments on infants (Lasch, 1923) and on young dogs (Adolph, 1943) indicate that an incompletely developed ability to reduce an excess water load by the renal route is not confined to newborn rats but occurs, though probably to a lesser degree, in other mammalian species.

Of the many factors which are known to influence the course of a water diuresis in adult animals, two only have been investigated in the present work. It was found that newborn rats absorbed water from the gastro-intestinal tract at a lower rate than adult animals of the same strain. This delay in the alimentary water absorption would by itself lead to a 'flattening' of the curve of water diuresis. However, it could be shown that this is not the only or even the most important factor which modifies water diuresis in newborn rats. (This seemed to be of importance since it is not known whether a similar retardation of alimentary water absorption occurs in other mammalian species. The relative immaturity of newborn rats will be remembered in this connexion.) Further investigation indicated that newborn rats were unable to excrete 'extra water' at the adult rate even after alimentary absorption had been completed. Determinations of the rate of extrarenal water loss of newborn rats—the second factor investigated—revealed that the rate of loss did not exceed that of adults at similar air temperature and showed also that, during the protracted period of alimentary water absorption, only a fraction of the initial water load was lost by the extrarenal route. Alimentary water absorption of the standard dose of water in newborn rats was shown to be completed in about 3 hr., and since it had also been shown that the urine volume did not increase during this time, it follows that any of the administered water residual after this period was lodged in the extra-alimentary tissues. In a series of rats kept at 30/31° C., it could be shown, for instance, that the mean residual (= tissue) water load after 3 hr. amounted to about 70 % of the dose of water administered and to about 60 % 5 hr. after the water had been given.

The latter finding would seem to have some bearing on a clinical problem. It is well known that infants within 2 or 3 days of birth are liable to develop a condition called 'oedema of the newborn' (Hallum, 1941). The incidence of this condition, which in Hallum's series progressed in some cases to generalized anasarca, was low (1·2 %) in children born at or near full term but comparatively high (13·4 %) in infants with a maturity of less than 36 weeks. It will also be remembered that oedema after parenteral fluid administration is frequently observed in newborn infants. It has been shown that newborn rats failed to give a diuretic response to water and that this failure produced a rise of the body water content which lasted for many hours. In other words, a dose of water which, owing to the quick alimentary absorption and quick renal excretion, increases the tissue water load of adult rats slightly and for a short period only (Heller & Smirk, 1932a) led to a state of 'oedema' in normal newborn rats. A comparison between the response of adult human beings and infants to the same relative extra water load may show whether the same factors contribute to the occurrence of oedema in newborn children.

SUMMARY

1. No evidence for an increase of urinary output could be obtained when a dose of water was placed in the alimentary tract of normally hydrated newborn rats and the urinary output measured for the following 5 hr.

2. The diuretic response to water (=water diuresis) is either very much diminished or completely absent in newborn rats.

3. The rate of gastro-intestinal absorption of water in newborn rats was estimated at two levels of air temperature (20/21 and 30/31° C.) and was found to be lower than in adult animals.

4. The body temperature, and therefore the metabolic rate, had little or no influence on the gastro-intestinal absorption of water in newborn rats.

5. The extrarenal water loss, per unit body weight, of newborn rats kept at 20/21° C. was only a small fraction of that of adult animals kept at the same air temperature. Newborn rats kept at 30/31° C. lost water extrarenally at about the same rate as adult rats at 20/21° C.

6. It could be shown that the slow alimentary absorption of water was not the only factor which prevented water diuresis in newborn rats. Newborn animals were unable to reduce an extra water load by renal excretion even when comparatively large amounts of water (about 70 % of the water administered) were demonstrably lodged in the extra-alimentary tissues.

REFERENCES

Adolph, E. F. (1943). *Physiological Regulations,* p. 266. Lancaster: The Jacques Cattell Press.

Brody, E. B. (1943). *Amer. J. Physiol.* **139**, 230.

Gullick, A. (1937). *Amer. J. Physiol.* **119**, 322.

Hallum, J. L. (1941). M.D. Thesis, Univ. of St Andrews. Cited by Young, W. F., Hallum, J. L. & McCance, R. A. (1941). *Arch. Dis. Childh.* **16**, 243.

Heller, H. (1944). *J. Physiol.* **102**, 429.

Heller, H. (1947). *J. Physiol.* **106**, 28.

Heller, H. & Smirk, F. H. (1932a). *J. Physiol.* **76**, 1.

Heller, H. & Smirk, F. H. (1932b). *J. Physiol.* **76**, 23.

Herrington, L. P. (1940). *Amer. J. Physiol.* **129**, 123.

Klisiecki, A., Pickford, M., Rothschild, P. & Verney, E. B. (1933). *Proc. Roy. Soc.* B, **112**, 496.

Lasch, W. (1923). *Z. Kinderheilk.* **36**, 42.

McCance, R. A. & Young, W. F. (1941). *J. Physiol.* **99**, 265.

Weiner, J. S. (1944). *J. Physiol.* **103**, 36 P.