Paradoxical Reduction in Tissue Hydration with Weight Gain in Neonatal Rabbit Pups

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Summary

We measured body hydration of healthy, term rabbit pups at birth and 72 hr of age. In addition, we measured hydration of individual tissues: skin, skeletal muscle, liver, and brain. At birth, the hydration of lean body mass, skin, and skeletal muscle, expressed as cc water per g fat-free dry weight (FFDW) was inversely proportional to birth weight. Low birth weight animals were significantly better hydrated than their larger littermates.

During the first 72 hr of life, the nursing pups gained an average 31.3% of birth weight. Simultaneously, lean body hydration decreased by 23% (5.48 to 4.86 cc/g FFDW). The hydration change varied substantially among organs. The greatest loss occurred in skin (24%). Skeletal muscle lost 5.8%, and the brain lost 3.1%.

When hydration at 72 hr was compared to weight gain and by inference fluid intake, a paradoxical result was obtained. Lean body, skin, and skeletal muscle hydration was inversely related to weight gain. The animals that gained the most weight retained the least water. In contrast, the hydration of brain and liver was significantly higher in animals that gained the most weight.

We conclude that the newborn rabbit is endowed with a reservoir of fluid at birth, predominantly stored in skin and skeletal muscle. The rate of release of this store depends on fluid intake. It is rapidly lost if ample fluid intake is provided. In conditions of restricted fluid intake, the release is substantially slower.

Speculation

At least two processes regulate tissue hydration in the newborn rabbit. The first, a function of growth, is responsible for the progressive accretion of intracellular water during gestation and postnatal development. It is illustrated by the increase in tissue hydration of brain and liver in well-nourished pups during the first 72 hr of life.

The second process may be under hormonal regulation on a time scale of hr to days. It accounts for the relatively high level of tissue hydration at birth, and provides a supply of water and sodium to maintain plasma volume during the first days of life, thereby preventing dehydration during this period of variable fluid intake.

The fetus undergoes a progressive decrease in total body hydration during gestation (2, 17, 24, 30, 37, 38), with alterations in partitioning of water: the intracellular fluid (ICF) increases and extracellular fluid (ECF) falls (1, 6, 11, 14, 15, 27). These processes continue in infancy and childhood until adult levels are attained (3, 38).

In the first days of extrauterine life, acute changes are superimposed on the long-term trends. Starved infants lose 7 to 11% of total body water in the first 72 hr of life (13, 30). The rise in ICF, occurring throughout fetal life, is temporarily reversed, and the newborn loses ICF for the first 72 hr before the level stabilizes and again resumes its increase (26). These statements describe

water balance in groups of neonates. However, individual infants may vary from this pattern. Operative delivery and perinatal events such as birth asphyxia may alter fluid and electrolyte balance in the neonate.

The tissue sources of the neonatal water loss have not been fully defined. Brain loses small amounts in newborn rodents (12, 18, 33, 34). One study described small water losses from rat skeletal muscle (20) whereas another found no muscle water loss at all in human newborns (28).

We undertook the current study in newborn rabbits to identify the tissue sources of neonatal water loss and to determine their relative contributions to total body water loss.

MATERIALS AND METHODS

Healthy timed gestation New Zealand White rabbit does were obtained from local commercial breeders. They were housed in standard cages with hay-filled nesting boxes and were fed Purina rabbit chow. We observed them frequently to determine the time of parturition.

As soon as possible after delivery, usually within 2 to 4 hr, the pups were weighed and marked, and one-half of the litter was sacrificed for initial hydration measurements. The remaining pups were returned to the mother who cared for them until 72 hr of age when they were reweighed and sacrificed. Some litters were sacrificed *in toto* at birth or at 72 hr.

The pups were killed by decapitation and exsanguinated. A piece of dorsal skin was immediately removed, weighed, and minced with scissors. Then, in rapid sequence, both quadriceps femoris muscles, the liver, and the forebrain were removed, weighed immediately, and minced. The remainder of the carcass was homogenized in a blender. The specimens were air-dried in a 93°C oven to constant weight. The tissue samples and an aliquot of the carcass specimen were then repeatedly extracted to constant weight with petroleum ether to eliminate fat. Fat and water content were calculated from differences in weight.

Groups were compared by Fisher's exact *t* test, and linear regressions were calculated using the method of least squares.

RESULTS

The mean birth weight of the term rabbit pups was 52.1 g (range, 29.5 to 74.5 g). Body fat and water composition at birth were direct functions of birth weight. Total body fat increased linearly with increasing birth weight ($n = 23$; $r = 0.920$; $P < 0.0001$). In contrast, the hydration of lean body mass, expressed as ml of water per g fat-free dry weight was an inverse function of birth weight (Fig. 1, $r = 0.570$; $P < 0.005$). The hydration of fat-free skeletal muscle ($n = 57$; $r = -0.435$; $P < 0.01$) and skin ($n = 37$; $r = -0.561$; $P < 0.001$) similarly decreased with increasing birth weight. Brain and liver hydration were unrelated to birth weight.

During the first 72 hr of life, the mean decrease in lean body hydration was 11.6%. Figure 2 compares lean body and individual

Fig. 1. The relationship between body hydration and birth weight at term.

Fig. 2. The hydration of lean body mass and of skin, skeletal muscle, brain, and liver at birth and 72 hr of age. Bars, S.E. Numbers in parentheses, number of animals in each group.

tissue hydration at birth and 72 hr of age. The extent of water loss varied among individual tissues. The greatest loss occurred in skin (24.2%) followed by skeletal muscle (5.8%) and brain (3.1%). Liver hydration did not change when the 72 hr group was considered as a whole.

The pups gained an average 31.3% of their birth weight (range, 19% loss to 74% gain). Weight gain varied considerably among litters. Some appeared very well cared for by the does. The pups were clean, and the nest was well made and well cared for. In such litters, particularly those with fewer than 8 pups, weight gain was uniformly high (40 to 60% of birth weight). Other litters appeared neglected by the does. Little effort seemed to have been expended on building or maintaining the nest. The pups became crusted with dried urine and were malodorous, and they lost weight. Variable weight gain also occurred in some of the larger litters which seemed well cared for. In one litter of 11 pups, 72-hr weight change varied from an 8% loss to a 50% gain.

At the time of sacrifice, the animals who lost weight or gained very little were uniformly deficient in subcutaneous and hepatic fat. Their gastrointestinal tracts contained little or no milk. In contrast, the gastrointestinal tracts of their more successful littermates were grossly distended with milk. The total body fat content

at 72 hr was directly related to weight gain ($r = 0.872$; $n = 28$; $P < 10^{-6}$). These observations suggest that weight gain is a reflection of milk intake and that low weight gain can most probably be ascribed to low intake of water and calories.

When 72-hr-old pups gaining more than 30 g were compared to those gaining less than 30 g (Fig. 3), significant differences are apparent. The anticipated increase in tissue hydration with greater weight gain was seen in brain and liver. However, lean body, skin, and skeletal muscle hydration had an opposite relationship. The animals that gained the most weight and by inference had the largest fluid intake retained the least water. Linear regression analysis further emphasizes the highly significant differences between hydration changes in brain (Fig. 4) and in lean body (Fig. 5) or skin (Fig. 6). Changes in skeletal muscle paralleled those in the total body and skin ($n = 31$; $r = -0.648$; $P < 0.0005$).

At the end of the 72-hr time period, four of the pups were clinically dehydrated. Their skin turgor was poor, and they were hypoactive or moribund. Table 1 shows the tissue hydration of these animals and compares them to groups that gained greater than and less than 30% of birth weight. They are also plotted on the regression curves as *filled circles*. As a group, they were less well hydrated than either weight gain group, but were nearer in

Fig. 3. Comparison of total body hydration and that of individual organs in animals gaining more than 30% of birth weight with those gaining less than 30%. Bars, S.E. Numbers in parentheses, number of animals in each group.

Fig. 4. The relationship between body weight gain and brain hydration at 72 hr of age. Regression calculated for normal pups only.

water composition to the high weight gain group rather than to the low weight gain group, the pups who were more similar in weight gain to the dehydrated animals.

DISCUSSION

The hydration of the newborn has been related to intrauterine growth. Widdowson (35) compared the largest and smallest piglets in three litters and found that the smallest contained a significantly

Fig. 5. The relationship between body weight gain and hydration of lean body mass at 72 hr of age. Regression calculated for normal pups only.

Fig. 6. The relationship between body weight gain and skin hydration at 72 hr of age. Regression calculated for normal pups only.

greater percentage of water than the largest. With correction for body fat, the same trend is found. Subsequent dessication studies in rats (22) and tracer experiments in human infants (5, 6) confirm that groups of growth-retarded infants have significantly greater hydration of lean body mass than appropriately grown controls.

We have extended these observations to show that lean body hydration at term in the rabbit is an inverse, linear function of fat-free body weight. One possible explanation of this observation would be inadequate removal of body fat. Adipose tissue has virtually no water content, and deposition of fat in the fetus is associated with decreasing total body water (36). Failure to remove fat completely would lead to underestimation of lean body and organ hydration. However, our measurements of body fat composition of the newborn rabbit are consistent with one other study (23) and slightly higher than two previously reported results (31, 35). Furthermore, we found clear correlations between birth weight and fat content and between 72-hr weight gain and body fat content. These results indicate that we obtained quantitative removal of body fat and make it unlikely that our hydration measurements are due to an inadequate removal of body fat.

The water content of the fetus falls progressively during gestation. Very early human fetuses weighing less than 20 g are composed of 91 to 95% water (36). At term, the water content has fallen to 82% of fat-free body weight (2, 36), similar to the 83.4% we have measured in the rabbit. This trend persists during infancy and childhood until the adult value of 72% of total body weight is attained (15, 37).

The smooth progress of antenatal changes in body water content is interrupted at birth by rapid fluid shifts during the transition from intrauterine to extrauterine life. Breast fed, term, human infants lose 7% of total body water during the first 72 hr of life (30), whereas starved prematures lose 11% in this period (13). This persistent fluid and electrolyte loss in the absence of intake has been ascribed to renal immaturity (19). However, subsequent work has shown that healthy newborns are capable of antidiuretic hormone and renal responses to moderate solute and volume loading (13, 25).

The water losses observed in starved infants persist if fluid is administered. Hansen and Smith (19) showed that when water was provided to starved, term infants, urinary water losses increased accordingly. Similarly, rates of total body water and weight losses are similar in starved and bottle-fed infants (26). In a group of prematures denied fluid for 72 hr, urine output peaked during the second day of fluid deprivation. These infants showed no clinical evidence of dehydration (4). During this time, plasma osmolality and sodium remained essentially unchanged (9). Thus, water and sodium are mobilized and excreted by the neonate even if intake is low.

There are clear differences between rabbits and human neonates in the degree of maturity at birth. In other respects, important similarities exist. *In utero*, both species are supplied with adequate fluid and electrolyte intake by the placenta and amniotic fluid and are protected against fluid losses by the uterine environment. Postnatally, in the normal situation, they are dependent upon the availability of milk to meet their fluid and electrolyte needs and lose substantial quantities of water to the environment.

In the current study, term nursing rabbit pups lost an average of 11.6% of total body water during the first 72 hr of life, a quantity comparable to measured losses in human infants. How-

Table 1. A comparison of the group of clinically dehydrated animals with those gaining less than 30% of birth weight and more than 30% of birth weight

	Mean wt gain (% of birth weight)	Tissue hydration (cc/g fat-free dry wt)			
		Total	Skin	Muscle	Brain
Dehydrated	-7.5	4.61 (0.15) ¹	4.42 (0.46)	5.37 (0.50)	8.27 (1.44)
Normal	15	5.24 (0.11)	5.11 (0.08)	5.66 (0.09)	8.99 (0.06)
	50	4.80 (0.08)	4.56 (0.07)	5.26 (0.10)	9.30 (0.05)

¹ Numbers in parentheses, S.E.

er, in contrast to the human babies who are also losing weight at this time, the rabbits gained relatively large amounts of weight. When we analyzed our results as a function of weight gain, and, by inference, fluid intake, an unexpected result emerged. The amount of neonatal fluid loss was inversely proportional to weight gain. The animals with the greatest fluid intake lost the largest proportion of fat free tissue hydration.

The water loss occurred to different degrees in different tissues. The greatest losses occurred in skin (24.2%) (to some extent the result of surface drying) and skeletal muscle (5.8%). Together these tissues comprise over 40% of body weight and 46% of body water in human newborns (37). Therefore, they are the major source of water loss. The brain of the human contributes 13% to total body weight and 17% to total body water (37). In contrast, the brain is only 2.3% of body weight in the newborn rabbit (22), and its contribution to total water flux is small. Rabbit brain loses 17% of its water content at 72 hr, comparable to previously reported values in rats (12, 34), mice (33), and rabbits (18). There are no similar studies in humans. However, intracranial volume has been calculated from head measurements in human infants at 72 hr of age and has shown a 7.5 to 9% decrease at this age (39). These larger changes in intracranial volume of human infants may be explained by alterations in the volume of cerebrospinal fluid. Our methods only assessed tissue hydration.

Concomitant with the decrease in lean body hydration during neonatal development is a change in partitioning of water between the intracellular and extracellular compartments. ICF gradually increases, and ECF falls (1, 6, 11, 14, 15, 27). This process also continues during infancy and childhood (3, 38).

Work by Maclaurin (26) in the human identified the ICF as the source of neonatal water losses. He measured thiocyanate space (an index of ECF), D_2O space (an index of total body water), weight, blood volume, and mean corpuscular hemoglobin concentration in a group of human infants during the immediate neonatal period. Total body water fell during the first 72 hr and then stabilized and began to rise by day 5. Parallel changes occurred in intracellular water. ECF rose slightly, and plasma volume remained stable. The decrease in intracellular fluid was reflected in red cell shrinkage identified by a rise in mean corpuscular hemoglobin. Others have documented significant decreases of cell water during the first day of life (6, 7). The development of legema in starved premature infants during the first day of life (32) may be another indicator of this fluid shift. Presumably, these infants are able to mobilize fluid from the ICF to the ECF. Movement into the vascular space, however, may be limited by low plasma oncotic pressure, a result of their low plasma protein concentration (29).

In Maclaurin's study (26), plasma osmolality did not change despite ongoing urine sodium losses, a net movement of water out of the intracellular space and slight expansion of the intracellular space. Davis *et al.* (9) obtained similar results. Thus, sodium accompanied the water shift. The source of this sodium is probably the intracellular space. Hazlewood *et al.* (21) summarized animal data indicating that intracellular sodium and chloride content decreased that of potassium at birth and that these relationships reverse during the first wk of life. They confirmed these results in newborn rats, documenting a ten-fold decrease in intracellular sodium and a concomitant decrease in tissue hydration.

We have demonstrated that these neonatal fluid shifts correlate with postnatal weight gain and fluid intake. Total lean body hydration and the water content of fat-free skin and skeletal muscle fall in inverse proportion to fluid intake. Low fluid intake results in retention of cell water whereas higher intakes permit rapid release. It would thus appear that neonatal intracellular water and sodium content are regulated by a mechanism which responds to fluid intake.

Other factors are also important. The skin, for example, appears moist and soft on day one and then dries and darkens in color. Most of the water loss from the skin is probably due to desiccation of the external, keratinized layers of cells after exposure to air. We suspect that this process is regulated by an homeostatic mechanism.

Changes in brain hydration differ from those of skin or skeletal muscle. Water content of brain at 72 hr of age was directly correlated with weight gain. In animals which gained over 30% of birth weight, brain water was identical to that of animals at birth. Brains of animals gaining less than 30% of birth weight contained less water than those of newborns ($P < 0.0001$) or their larger littermates ($P < 0.001$). Similarly, liver hydration was greater in animals who gained more than 30% of birthweight. It would appear that the regulators of brain and liver hydration differ from those regulating skin and skeletal muscle water, the main determinants of total body water.

Starved human neonates lose 7 to 11% of total body water in the first 72 hr of life (13, 39), yet show no clinical signs of dehydration save for moderate oliguria (4). Older infants with a similar degree of acute water loss are usually clinically dehydrated and sick. A similar observation in newborn pigs has been made by Gentz *et al.* (16). They were investigating the metabolic effects of starvation and noted that plasma volume was well maintained for 96 hr in piglets starved from birth. Those allowed access to feedings for 24 hr or longer before starvation showed significant decreases of plasma volume within the first 48 hr of starvation. We would postulate that the latter group of animals had already mobilized a substantial portion of its water reserves during the period of feeding and was thus less well prepared for a period of fluid deprivation.

It appears that cell water and sodium content can be controlled in the perinatal period. There is an intracellular reservoir of fluid and electrolyte at birth. This fluid is released rapidly if sufficient fluid intake is available. In the absence of adequate intake, it is released more slowly, perhaps as a means to protect the circulating blood volume and thereby to maintain tissue perfusion. Despite measured "dehydration" of body tissues, clinical signs of dehydration usually do not become evident. However, with very low intake, neonatal water losses may ultimately exceed the compensatory capacity of tissue reserves. We found four of the pups to be clinically dehydrated, and their tissue hydration to be less than that of their healthy counterparts. It is of interest that their tissue hydration more closely resembled that of pups with large weight gains, rather than pups who had similarly low gains or losses.

There is an interesting maternal parallel to gestational and neonatal fluid shifts. During gestation, there is a significant expansion of maternal body fluid, often associated with the onset of clinical edema. This change is promptly reversed in the early postpartum period (8). Inasmuch as mother and fetus share a similar hormonal milieu, the search for the hormonal mediators of these fluid shifts might profitably be pursued within the group of hormones that are elevated in late pregnancy and that fall during the immediate postnatal period.

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41. The authors thank Dr. H. William Taeusch, Director, Joint Program in Neonatology, for his encouragement and support during this research and for thoughtful review of the manuscript. Dr. Clement A. Smith also provided helpful suggestions during its preparation.
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43. Received for publication February 7, 1979.
44. Accepted for publication January 14, 1980.