Changes of Plasma Osmotic Pressure during Lactation in Rats

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ABSTRACT. It is known that blood and plasma volume increase during lactation. The present paper examines whether an increase in plasma volume is accompanied by the change in plasma composition or attributed to hydro-dilution. Six dam-nursed pups and six dam-removed pups housed individually were designated as lactating rats and control rats, respectively. The plasma osmotic pressure and hematocrit value (Ht) were measured in the rats on days 3, 5, 7, 10, 13 and 18 of lactation. The total plasma protein (TP) and serum sodium concentration were also measured as they are factors affecting the plasma osmotic pressure. In addition, milk yield was estimated by the Morag technique. On day 5 and after day 10, the osmotic pressure of the lactating rats was found to be significantly lower than that of the control rats. The serum sodium concentration (days 5 and after day 10) and Tp values (days 3, 10 and 18) of the lactating rats were significantly lower than those of the control rats. Except on day 5, the Ht values of the lactating rats were significantly lower than those of the controls. During the period between days 3 and 10, milk yield was increased and it become steady (18 g/12 hr) on days 10 and 18. On and after day 10 when rats secreted a large amount of milk, it is considered that a decrease in the plasma osmotic pressure was mainly attributed to the reduction of sodium concentration by hydro-dilution. The Ht values indicate that an increase in blood volume is mainly through plasma volume rather than blood cell volume in lactating rats.—KEY WORDS: hydro-dilution, lactation, milk yield, plasma osmotic pressure, rat.


Lactation is accompanied by striking increases in water, food consumption and body weight [19, 22]. Cripps and Williams [8] reported that absolute absorptions of leucine and glucose increase during lactation in rats. Dams that receive suckling stimulation from their pups consume significantly more water and food, and gain more weight than dams without pups [18]. The increased intake of water provides milk with an adequate amount of water for the young, and metabolism of dams is activated through milk production.

Some studies have demonstrated that dam’s blood volume increases during pregnancy [1–4, 7, 13, 14, 20]. This increase is associated with an elevation in cardiac output [16]. It has also been found that cardiac output increases during lactation [6, 9, 10–12], and that blood and/or plasma volumes increase in rats [4, 5] and cows [20, 21] during lactation. However, the mechanism for the increase in plasma volume in lactation has not been clarified. Bond [5] found that hemoglobin concentration, total hemoglobin and erythrocyte volume in circulation increased during lactation in rats. However, the extent of increase in blood cell volume and plasma volume is not known. In addition, it is not known whether the plasma osmotic pressure changes through milk production. The present work examined whether or not an increase in plasma volume was accompanied by the change in plasma composition induced by hydro-dilution.

MATERIALS AND METHODS

Animals: Wistar strain rats, first lactation, were housed individually under controlled conditions of temperature (22°C) and lighting (12 hr light) in a well ventilated room. Water and diet (CRF-1, Charles River Japan Inc., Kanagawa) was available ad libitum throughout the experimental period. The day of parturition was designated as day 0 of lactation. The rats were divided into two groups: (1) Lactating groups, litter size adjusted to twelve pups at parturition and (2) Control groups, litter removed from dams following parturition. Both groups (6 animals per group) were studied on days 3, 5, 7, 10, 13 and 18 of lactation (from 9:00 to 12:00 hr). Milk yield was measured by the Morag technique [17]. The method used was as follows. The pups were separated from the dams for 12 hr and were then allowed to suck for about 2 hr. The increase in litter weight during suckling was used to estimate milk secretion.

Sample collection: The rats were anesthetized with pentobarbital sodium (Abbott Lab., Illinois, U.S.A.). 5 mg/100 g body weight, given intraperitoneally after the estimation of milk secretion. Blood samples were taken from the V. Cava caudalis, some of these were measured for Ht and then stored in heparin coated tubes or in test-tubes. After centrifugation, the serum and plasma samples were stored at −20°C until assay.

Assay: The plasma osmotic pressure was determined by the freezing point depression methods using an Osmometer OMS801 (Vogel Co., Germany). TP was determined using Bio-Rad (Bio Lab. Inc., Osaka) protein assay using Control Serum 1 (Wako Pure Ind., Osaka) as the standard. Serum sodium concentration was obtained by electrodes using STAX-1 (Techno Medica Co., Kanagawa).

Statistical analysis: Data was expressed as the means ±
S.D. The non-paired Student t-test was used for the analysis of statistical significance. Levels of significance are indicated with asterisks, * p<0.05, ** p<0.01, *** p<0.001.

RESULTS

Daily changes of pup weights and dam milk yields are shown in Fig. 1. Pup weights on days 3 and 5 of lactation remained 7 and 8 grams, respectively, and after that they increased. During the period between days 3 and 10 of lactation, milk yield was increased. On days 10 and 18 of lactation, it remained about eighteen grams per twelve hours.

The time course of changes in the plasma osmotic pressure is shown in Fig. 2. On day 5 and after day 10 of lactation, the plasma osmotic pressure in the lactating rats

Fig. 1. Daily changes in pups weight and dam's milk yield during lactation. Dams milk yield (■) and pups weight (○).

Fig. 2. Changes of the plasma osmotic pressure during lactation in the rats. Lactating group (■), control group (○). Each symbol represents the mean value for 6 rats in each group. Levels of significance were indicated by symbol *, P<0.05; **, P<0.01; ***. P<0.001.

Fig. 3. Changes of serum sodium concentration (A) and total protein (TP, B) during lactation. Lactating group (■), control group (○). Each symbol represents the mean value for 6 rats in each group. Levels of significance were indicated by symbol *, P<0.05; **, P<0.01, ***. P<0.001.

Fig. 4. The time course of changes in Hematocrit values (Ht) during lactation. Ht values were significantly lower in lactating animals (■) than in control animals (○). Each symbol represents the mean value for 6 rats in each group. Levels of significance were indicated by symbol *, P<0.05; **, P<0.01; ***. P<0.001.
was significantly lower than that in the control rats. Serum sodium concentration and TP were measured on each experiment day as these affect the plasma osmotic pressure. The sodium affects the plasma osmotic pressure as an electrolyte and TP is a type of colloid osmotic pressure. As shown in Fig. 3A, there was a significant difference in the serum sodium concentration between the lactating and control rats on day 5 and after 10 of lactation. The results of the plasma osmotic pressure and serum sodium concentration were the same. Figure 3B shows the changes in TP during lactation. On days 3, 10, and 18, TP was significantly lower in the lactating animals than in the control animals.

Ht was significantly lower in the lactating rats than in the control rats during the experimental period except on day 5 of lactation, although blood cells did not affect the plasma osmotic pressure (Fig. 4).

**DISCUSSION**

Pup weight gains observed in this study were similar to those reported by Sakanashi et al. [22], Ota [19] and Hanwell and Linzell [11]. Milk yields were considered to have a relation to the plasma osmotic pressure. A report on the day rhythms in Ht [23] is included. The studies were performed from 09:00 to 12:00 hr to prevent fluctuation of Ht value by the daily rhythm.

The experiment was assigned to clarify postpartum changes of the plasma osmotic pressure through milk production. After day 10 of lactation, the plasma osmotic pressure of the lactating rats was found to be significantly lower than that of the control rats. The result of serum sodium concentration was similar to that of the plasma osmotic pressure but the result of TP was a little different from that of the plasma osmotic pressure. Therefore, it is considered that the decrease in the plasma osmotic pressure could be mainly attributed to the reduction of sodium concentration in the circulation. Atherton et al. [1] found that changes in the plasma osmotic pressure were similar to those in serum sodium concentration in pregnancy, and that both values decreased with progression of pregnancy until the second trimester. They suggested that the decrease in the plasma osmotic pressure resulted from a decrease in plasma concentration that could be induced by hydro-dilution. During lactation, it might be speculated that plasma is diluted with water in a similar way to pregnancy.

A number of reports on vasopressin, the hormone for water retention in lactation are available. Lightman and Young [15] studied mRNA of vasopressin in rats, and found that vasopressin mRNA in rats on day 10 of lactation was significantly elevated compared to virgins. Zingg and Lefebvre [24] determined the level of rat hypothalamic oxytocin and vasopressin mRNA during pregnancy and lactation using densitometric hybridization assay. During lactation, vasopressin mRNA reached the level exceeding the virgin control. The results of the present work suggest that water retention in the lactation may occur mainly in vasopressin. Ht of the lactating rats was significantly lower than that of control rats. It is likely that the blood volume increases mainly due to plasma volume rather than blood cell volume. However, Bond [5] found that Ht did not significantly differ from the virgin value. The discrepancy between Bond's findings and this paper might be attributable to litter size. Bond did not adjust to litter size (average litter size was 6.5 in the range of 2-11). In the present work, the litter size was adjusted to twelve pups with the aim of maintaining this strain of rats from the Imamichi Institute for Animal Reproduction.

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**REFERENCES**


