CLXXXII. THE EFFECT OF WEANING UPON THE EXCRETION OF CALCIUM IN THE URINE OF LACTATING RATS.

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Goss & SCHMIDT [1930] observed a marked loss of calcium from the calcium stores of the body of the rat during lactation. They did not separate the urine and faeces in their work, nor did they study the effect of weaning upon the excretion of calcium. It has been shown histologically by Selye [1934] and Jeffers [1935] that practically all the milk disappears from the mammary gland of the rat within 6-8 days after weaning, whilst on the second day after weaning the glands are quite distended with milk. Maeder [1922] claims that the secretory activity of the mammary gland of the rat is not noticeably diminished until the third day after weaning. Weatherford [1929] observed a decrease in the secretory activity of the mammary gland after the tenth day of lactation. This corresponded to the peak of the growth period of the young and the eruption of teeth. Involution of the mammary gland occurred more readily after the fourteenth day of lactation.

The present investigation was undertaken to study the effect of weaning upon the excretion of calcium in the urine of rats at different stages of lactation.

Methods.

Adult female rats were obtained from the colony on the day of birth of the litter; the size of the litter was reduced to 6 in number. The young were allowed to suckle the mother for a definite number of days and then weaned. The mother was placed in a Hopkins metabolism cage and the excretion of calcium in the urine determined for a period of 12 days. Urine was collected over a 2-day period. The whole specimen of urine was ashed on a heated sand-bath in the presence of concentrated nitric acid. The ash was dissolved in dilute hydrochloric acid and made up to a definite volume and calcium was determined on a suitable aliquot by the micro-method of Clark & Collip [1925].

Results.

The results are shown in Table I. The values are based on the average excretion of 4 rats in each group. The first column shows the effect of parturition on the excretion of calcium in the urine. These rats were placed in the metabolism cages 6 days before delivery. It is seen that the excretion of calcium in the urine of these rats is very small, amounting to approximately 0.1 mg. per rat per day. No significant increase occurred as a result of parturition which took place on the sixth day. Furthermore, no increased excretion of calcium occurred in the urine of these rats as long as the mother was allowed to suckle the young. It was not feasible to follow the calcium excretion of these rats for a longer period, since the young were not satisfactorily looked after by the mother in the open metabolism cage without any nesting material. However, the excretion

Table I. *The effect of weaning upon the excretion of calcium in the urine of lactating rats.*

(Expressed in mg. per rat per day; average of 4 rats.)

Days	Before and after parturition	Weaned at birth of litter	Weaned after 5 days' lactation	Weaned after 10 days' lactation	Weaned after 15 days' lactation	Weaned after 20 days' lactation	Weaned after 25 days' lactation	Mammary glands removed, 10 days' lactation
2	0.12	0.45	0.56	2.39	2.48	0.55	0.53	0.13
4	0.07	0.35	0.71	6.74	3.47	1.69	0.88	0.04
6	0.03*	0.04	0.04	0.05	0.10	0.10	0.05	0.08
8	0.06	0.20	0.07	0.03	0.05	0.06	0.05	0.04
10	0.04	0.14	0.04	0.02	0.04	0.08	0.02	0.09
12	0.11	0.15	0.05	0.02	0.05	0.08	0.04	—

* Day of parturition.

of calcium in the urine of this group serves as a control for the other groups as it shows a low excretion of calcium in the urine during suckling.

In the second column the effect of weaning at the birth of the litter is shown. It is seen that the calcium excretion in the urine is three to five times that found in the control group during the first 4 days. After this time, the urine calcium is quite within the limits of that found normally. When the young are allowed to suck for 5 days and then weaned, as shown in the third column, a slightly higher value is found for the first 4 days. When lactation is continued for 10 or 15 days (columns 4 and 5) a very markedly increased excretion of calcium in the urine occurs. It is noted that the excretion is greater on the third and fourth days of weaning than on the first and second. Thereafter the calcium excretion is similar to the control values. When lactation is continued for 20–25 days before weaning (columns 6 and 7), the values are decidedly lower than those found on the tenth and fifteenth days of lactation. The last column shows the results of removing all the mammary tissue of rats which had been lactating for 10 days; no increase in calcium excretion in the urine was observed. This shows that the calcuria of weaning is due to resorption of the milk from the mammary gland.

A group of 4 rats were weaned after 10 days' lactation; on the third day of weaning the serum calcium showed a slight increase. The average value was 12.2 mg./100 ml., whilst the value for non-lactating female rats was 10.4 mg./100 ml.

DISCUSSION.

The calcuria observed upon weaning lactating rats is of interest in that the animals do not appear capable of immediately restoring the depleted calcium supplies. Goss & Schmidt [1930] found that they could markedly deplete the bones of calcium by repeated pregnancies in rats on a low-calcium diet. Similar results have been reported for cows. The calcuria observed under these circumstances resembles the lactosuria observed by Brock and Hubbard [1935] and others in human subjects during the last month of pregnancy and for a time after parturition. The markedly increased excretion of calcium after 10 days' lactation agrees with the observations of Weatherford [1929] in that this time appears to be the peak of the secretory activity of the mammary gland. Cox [1936] finds that he can obtain a greater yield of milk from rats which have been lactating for 12–14 days.

An attempt was made to prolong the period of increased calcium excretion by the administration of the anterior pituitary hormone "prolactin". However,

this treatment neither prolonged the period of calcuria nor caused a greater increase in the calcium in the urine than was obtained normally in rats which had been lactating for 10 days. Selye *et al.* [1933] report that ovariectomy after 26 days' administration of the anterior pituitary-like hormone of the placenta initiates lactation in non-pregnant female rats. However, upon repetition of this experiment, no increase in excretion of calcium occurred although slight evidence of milk was seen in the glands histologically. This negative result is undoubtedly due to the fact that it is very difficult to stimulate the mammary glands of the rat to a condition comparable with that found after parturition.

SUMMARY.

The weaning of lactating rats causes an increased excretion of calcium in the urine which is maintained for 4 days. A gradual increase in the calcuria was found from parturition until the tenth day of lactation; after this the calcium excretion was diminished. Removal of all mammary tissue from rats lactating for 10 days resulted in no increase in excretion of calcium. It is concluded that the calcuria observed upon weaning lactating rats is due to the resorption of milk from the involuting mammary gland.

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REFERENCES.

- Brock & Hubbard (1935). *Amer. J. dig. Dis. Nutrition*, **2**, 27.
Clark & Collip (1925). *J. biol. Chem.* **63**, 461.
Cox (1936). *J. biol. Chem. Proc.* xxiii.
Goss & Schmidt (1930). *J. biol. Chem.* **86**, 417.
Jeffers (1935). *Amer. J. Anat.* **56**, 257.
Maeder (1922). *Amer. J. Anat.* **31**, 1.
Selye (1934). *Amer. J. Physiol.* **107**, 535.
— Collip & Thomson (1933). *Proc. Soc. Exp. Biol. N.Y.* **30**, 588.
Weatherford (1929). *Amer. J. Anat.* **144**, 199.