there is an increase in cardiac output proportional to the reduction in hematocrit(1,5, 15). Nevertheless, the circulatory adjustments are insufficient to maintain a normal oxygen delivery to the body tissues. In the anesthetized dog with constant blood volume the amount of oxygen arriving at the tissues is at its maximum only when the hematocrit is normal; it is reduced both in polycythemia and in anemia(4,15). In the present study the increase in output due to the anemia alone was insufficient to maintain the delivery of oxygen to the tissues at the control value; only when blood volume was increased as the hematocrit decreased did the output increase sufficiently to deliver the same number of red cells to the periphery. Thus, in spite of the similar changes in blood viscosity, it was only when the filling pressure of the ventricles was augmented that the heart was enabled to increase its output sufficiently to maintain oxygen transport to the tissues.

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Received May 4, 1964. P.S.E.B.M., 1964, v117.

Effect of Sodium Citrate on Milk Yield in Rats.* (29663)

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The existence of an inhibitory factor in muscle tissues was reported(1,2). The presence of this factor prevented muscular contraction even in the presence of adenosine triphosphate (ATP). By adding a calcium solution, however, the inhibition was prevented and contraction occurred. It was suggested that the calcium released by nerves during their stimulation in some way negated the inhibitory factor thus making possible the contraction of the innervated muscle(3). In the mammary gland, myoepithelial cells have been observed lying in close contact with the epithelium of the alveoli and ducts. It has been suggested that these cells have contractile properties which respond to the stimulus of oxytocin causing the milk in the alveoli to be discharged into the larger ducts and thus becoming available to the nursing young (4).

Evidence that myoepithelial cells are contractile comparable to smooth muscle cells is based on visual examination of the exposed

* Contribution from Mo. Agr. Exp. Sta. Journal Series No. 2713. Approved by the Director. This investigation was supported in part by research grants from USPHS.

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gland rather than on direct measurements.

Following thyroparathyroidectomy of lactating rats, it was shown that the litter weight was reduced and very little milk could be removed on day 14 of lactation(5). While the reduction in milk secretion may have been due, primarily, to the low serum calcium level due to parathyroidectomy, the rats at the end of the test milking showed the presence of considerable amounts of milk which was not removed by oxytocin during the 30 minute nursing procedure. This observation suggested that the low serum calcium level might be interfering with the contractile response of the myoepithelial cells to oxytocin as well as interfering with milk secretion.

The present study shows that low serum calcium levels do, in fact, interfere with the contractility of these cells and the removal of milk by nursing young.

Materials and methods. In previous studies with 18 to 22 g mice, it was shown that 0.05 ml of a 25% sodium citrate solution caused violent convulsions in 58% of the mice, obvious degrees of convulsions in 99% but no deaths(6). In lactating rats of the Sprague-Dawley-Rolfsmeyer strain, it was observed that 1.25 ml of the 25% citrate solution was effective in inducing a short period of convulsions (tetany) when injected intraperitoneally. The maximum effect was reached in 10 to 15 minutes, then the animals gradually recovered. The above level of sodium citrate was used as described.

A group of 21 lactating rats was housed, maintained on food and water and their milk yields determined on days 14, 16, 18, and 20 of lactation as described previously(7). Two USP units of oxytocin (OXT) rather than one unit normally administered, was given to the animals prior to the time of the test milkings. On days 16 and 20 of lactation, convulsions were induced in 15 animals. At the time of maximum effect, OXT was injected into the mothers, and the young were allowed to nurse for exactly 30 minutes. By the end of the nursing period some of the animals had completely recovered, but many still showed tremors of their legs. Six animals served as controls and their milk yields were estimated without treatment except for OXT.

TABLE I. Effect of Sodium Citrate on Milk Yield of Rats.

No. of animals	Milk yields (g) on:* Day 14	Day 16	Day 18	Day 20
	Normal controls			
20	14	16	11	23
21	11	17	17	19
22	13	14	10	†
23	8	20	23	10
24	14	17	23	12
25	14	13	25	15
Mean	12.3	16.2	18.2	15.8
S.E.	1.00	1.00	2.64	2.36
	Experimental			
	Normal	Treatment	Normal	Treatment
2	8	0	10	6
3	13	6	15	9
4	9	0	11	0
5	11	7	20	18
6	13	5	8	0
7	12	0	15	1
8	13	13	15	9
9	15	13	10	0
10	13	19	14	3
11	10	11	14	3
13	16	0	17	7
14	10	4	11	3
15	10	5	8	3
16	18	14	10	17
17	15	12	13	10
Mean	12.4	7.3	12.7	5.9
S.E.	.73	1.51	.89	1.51
P‡	>.9	<.01	>.02 <.05	<.01

* Litter was reduced to 6. Treated animals received 1.25 ml of 25% solution of sodium citrate.
† Animal died.
‡ Student's "t" probability, significant differences of the mean from control animals.

Results. Normal milk yield was obtained from all rats on day 14, the 6 normal animals yielding a mean of 12.3 g and the experimental group of 15 producing a mean of 12.4 g. On day 16, the control group produced a mean milk yield of 16.2 g but the animals administered sodium citrate produced a mean of only 7.3 g, a reduction of 41% from their own 14 day yield and of 55% from the control group. Four animals that produced from 8 to 16 g on day 14 failed completely to yield milk on day 16 after convulsions were induced (Table I). Most of the other rats showed a marked reduction in yield.

On day 18, the milk yield of all rats was

again determined without treatment except OXT. The 6 control animals yielded a mean of 18.2 g and the experimental group yielded a mean of 12.7 g. The lower production of the experimental group may have been due to the residual effect of the sodium citrate upon milk secretion following the test on day 16.

On day 20, the control group produced a mean milk yield of 15.8 g. Whereas the experimental group produced a mean of 5.9 g, a reduction of 54% of their own 18 day production and of 63% of the production of the control group. Three animals producing 8 to 11 g failed to yield milk and all but one animal showed lower production compared with their 18 day production.

Discussion. It is recognized that calcium is present in blood in 3 fractions: the nondiffusible or protein-bound-calcium, the ionized calcium and calcium that is complexed to phosphate, bicarbonate or citrate. It is believed that only a small amount of calcium in plasma is in the form of calcium citrate complex and that the citrate complex is completely dissociated in the form of: $Ca(CaCit.)_2 \rightleftharpoons Ca^{++} + 2\ CaCit.^-$ (8,9). The following important functions of calcium in body tissues are well established: maintenance of normal cardiac rhythm, regulation of membrane permeability, maintenance of milk production, and regulation of normal muscular and nervous excitability. A condition of parturient paresis may develop in dairy cows due to a lowering of blood calcium and phosphorus as a conseqeunce of intensive milk production (10,11).

The failure of muscles to contract *in vitro* in the absence of calcium, but to contract when a calcium solution was added, led authors to postulate the existence of an inhibitory factor in skeletal muscles which could be blocked by the action of calcium (1,2).

The present experiment indicates that the myoepithelial cells surrounding the alveoli and ducts in the mammary gland which contract normally upon the stimulation of nursing, releasing oxytocin, require the presence of ionized calcium in the blood plasma similar to the requirements of smooth muscles for contraction. The variation in the response to sodium citrate in individual animals may have been due to variation in the level of ionized calcium present, to the time required for gradual dissociation of calcium citrate, and to the rate at which calcium might be mobilized from the bone to restore normal calcium levels in the plasma. The observation that a total block occurred in some animals whereas a partial block occurred in others is related also to the 30 minute nursing period during which various degrees of recovery occurred.

While the lack of ionized calcium in the blood plasma is believed to be primarily responsible for the reduction in milk yield observed, it is possible that the convulsions induced in the dams may have stimulated the release of adrenaline which is known to cause a contraction of the blood vessels of the mammary gland and thus prevents oxytocin acting on the myoepithelial cells.

The decline in milk yield after parathyroidectomy following the decline in ionized plasma calcium may be due to 2 effects upon the mammary apparatus, (1) a reduction in synthesis of milk, and (2) interference with removal of the milk secreted.

A third effect has been suggested (12) namely, on the composition of the milk. It was reported that the calcium content of rat milk was increased markedly with an increase of total solids. Unfortunately, the method of obtaining the milk sample for analysis was not reported. In the light of the present work, it is unlikely that a truly representative sample of milk was obtained for analysis.

It is possible in cows in early lactation that the reduction in plasma calcium due to intense milk secretion even though not sufficient to cause parturient paresis may be sufficient to interfere with the normal contractility of the myoepithelium and thus limit milk yield.

Summary. The milk yield of rats was determined on days 14, 16, 18 and 20 by separating the litters from their dams for 10 hours, then injecting 2 USP units of oxytocin and allowing the young to nurse for 30 min-

utes. Immediately prior to test milking on days 16 and 20 an experimental group of 15 rats was injected with 1.25 ml of 25% sodium citrate to cause convulsions (tetany) due to the decrease in ionized plasma calcium. The milk yield of these rats was greatly reduced in comparison with their own previous production and in comparison with the milk yield of the control group on the same day. It is suggested that the contractility of the mammary gland myoepithelial cells by oxytocin is greatly reduced by the lack of plasma calcium. Thus the decreased milk yield of parathyroidectomized rats is due to a reduction in the synthesis of milk and interference with the removal of the milk secreted.

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Received May 25, 1964. P.S.E.B.M., 1964, v117.

Effect of Cysteine on Formation of Streptolysin S by Group A Streptococci.*† (29664)

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It has been shown(1,2,3) that serum albumin, Tween 40 and Triton X-205 induced the formation by resting group A streptococci, of an oxygen-stable hemolysin indistinguishable from the serum or RNA hemolysin (streptolysin S) described by Todd(4) Okamoto(5) and Bernheimer(6). Studies employing chromatographic and electrophoretic techniques have indicated that streptolysin S of group A streptococci is a complex of a nonspecific carrier, such as RNA, albumin or tween, and a specific hemolytic moiety(2). The hemolytic moiety which is synthesized by both growing and resting streptococci is destroyed by papain and chymotrypsin and was postulated to be a protein(3,6). More recently, it was shown that streptolysin S induced by yeast RNA is a complex of an oligonucleotide and a peptide. The latter is composed of 10 amino acids(7,8). For optimal formation of the RNA hemolysin by resting streptococci maltose, Mg^{++} and a fraction of yeast RNA resistant to pancreatic ribonuclease are necessary(9). On the other hand, glucose, Mg^{++} and cysteine are essential for formation of the serum, albumin or tween hemolysins(1,2).

The purpose of the present study was to investigate the effect of sulphydryl compounds on formation of the RNA hemolysin. Also it will be shown that the inhibition of hemolysin formation caused by various amino acids analogues is not effective in the presence of sulphydryl compounds.

Materials and methods. Streptococcal strains and culture media. Most of the experiments described were performed with a group A streptococcus type 3 strain S 84

* Supported by a research grant from Israel Foundation Trustees.

† This work was done under a fellowship from Abner D. Ungar Fellowship Fund.