

ditions of oviduct secretions from the sow, ewe and rabbit were found to increase O₂ uptake by 18, 11 and 12% respectively over the maximum rate obtained with bicarbonate suggesting the presence of additional stimulants in female tract secretions.

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A Transient Fall in Pressure Precedes the Characteristic Intramammary Pressure Rise Following Mechanical Stimulation of the Mammary Gland.* (32527)

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The myoepithelium of mammary glands in goats(1), mice(2), rabbits(3,4) and rats(5) contracts when mechanical stimulation is applied to the skin overlying the mammary gland or directly to the exposed mammary tissue.

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This contraction causes a rise of intramammary pressure similar to that produced by oxytocin. It has been suggested that in some species stimulation of the myoepithelium by the kneading and butting activity of the suckling young may represent a subsidiary mechanism for milk ejection which reinforces the action of oxytocin(3,5). In the course of a study of the interaction of nervous, hormonal and mechanical influences on intramammary pressure, we noticed that mechanical stimulation of the rat mammary gland caused a transient fall of intramammary pres-

sure below the baseline. This preceded the characteristic pressure rise.

Materials and methods. Observations were made in 15 primiparous Wistar rats lactating 7-18 days and in one New Zealand rabbit lactating 10 days. Each animal was anaesthetized with urethane (1.4 G/100 G, i.p.) and was placed on a heated blanket in a supine position. An abdominal mammary gland was cannulated with a stainless steel cannula which was connected by wide bore polyethylene tubing to two Statham P23 pressure transducers, one of high, the other of low sensitivity. The transducer outputs were recorded on a Grass Model 5 Polygraph and calibrated using a mercury manometer. The transducers were filled with 0.9% saline and the rest of the system with citrated cow's milk. A 3-way stopcock was placed between the transducers and the mammary gland. This allowed the addition of milk to the system from a pipette.

Mechanical stimulation of the mammary gland myoepithelium was effected by single taps to the overlying skin with the flat edge of a scalpel handle. Intrajugular injections of oxytocin (0.5-1.0 mU in 0.05 ml saline) were also used to induce myoepithelial contraction. In some rats the cannulated mammary gland was denervated by section of the L IV-L VI segmental nerves between the mammary gland and the vertebral column. In others a solution of 1% lignocaine ("Xylocaine," Astra Hewlett) was used instead of milk to fill the mammary gland.

Results. The intramammary pressure responses of rats to mechanical stimulation were not detectable until 0.4-0.6 ml of milk had been added to the gland *via* the intraductal cannula. Responses were elicited in 14 out of 15 rats and in the one rabbit tested. The initial sharp rise in pressure due to the tap stimulus was followed by a decrease in pressure of 1-5 mm Hg, which reached its maximum value 0.5-2.2 seconds (though usually 1.0-1.5 seconds) after the tap. After this brief reduction in pressure, there followed a rise which reached its peak in about 3-4 seconds, returning to the resting pressure in 10-45 seconds. A variety of such responses is shown in Fig. 1A.

A sharp reduction in intramammary pres-

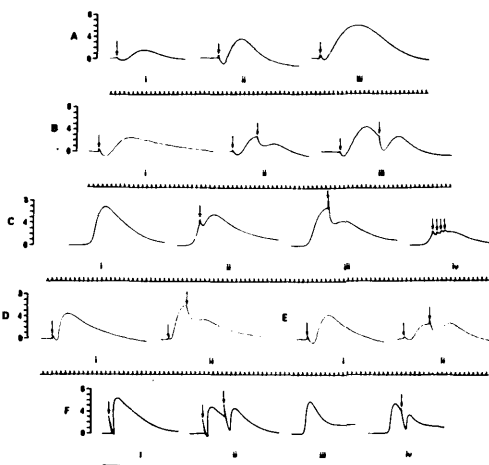


FIG. 1. Intramammary pressure responses of rats (A - E) and a rabbit (F) to mechanical stimulation effected by a "tap" of the flat edge of a scalpel handle applied to the skin overlying the gland. A: Responses to single tap in 3 different glands. B: Responses to single (i) and successive (ii, iii) taps in same gland. C: Responses to 0.5 mU oxytocin in same gland (i -iv) as affected by single (ii, iii) or multiple (iv) taps. D: Responses in denervated glands. E: Responses in glands filled with lignocaine instead of milk. F: Responses in rabbit gland to single tap (i), two taps (ii), 1 mU oxytocin (iii), oxytocin and tap (iv). Pressure in mm Hg. Time scales in seconds.

sure occurred when the mechanical stimulus was applied during an existing elevation of intramammary pressure, induced either by a previous tap (Fig. 1B) or by oxytocin injection (Fig. 1C). The reduction in intramammary pressure so caused was much larger than that seen when the stimulus was applied to a resting gland, and was often large enough to reduce the pressure to the resting value. There usually followed a second rise in intramammary pressure, but in no instance was there any indication of summation of the responses to any two mechanical stimuli or to a combination of mechanical and oxytocin stimuli. Repeated mechanical stimuli applied to the mammary gland could, in fact, be shown to interfere with the normal development of an oxytocin-induced rise of intramammary pressure (Fig. 1C iv). Similar negative and positive phases of the intramammary pressure response were seen in rats after denervation of the mammary gland (Fig. 1D) and after introduction of lignocaine into the gland (Fig. 1E).

In a single rabbit, there occurred a similar

reduction of intramammary pressure immediately after mechanical stimulation. The reduction in pressure again was particularly marked when the mechanical stimulus was applied during an existing rise in intramammary pressure provoked by a previous mechanical stimulation or oxytocin injection (Fig. 1F).

Discussion. A reduction in intramammary pressure immediately following mechanical stimulation of the gland was observed consistently in our animals. The failure of previous authors(3,4) to detect the phenomenon probably was because they did not use isometric recording techniques. Grosvenor(5) used a recording system similar to that used in the present study, though it was of rather greater inertia. Despite this, however, re-examination of the records made in Grosvenor's study(5) indicates that the reduction in intramammary pressure often followed tap stimuli. The phenomenon was less marked, as would be expected from a system with a lower frequency response.

A hypothetical scheme is put forward in Fig. 2 to explain the observed pressure

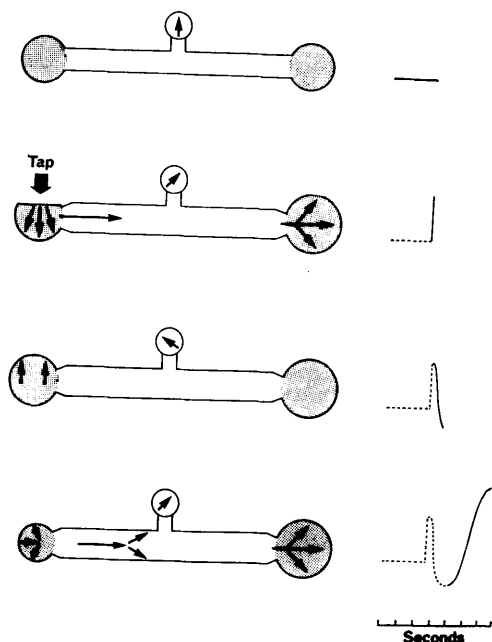


FIG. 2. Hypothetical scheme to explain negative and positive phases in the intramammary pressure response to mechanical stimulation. For details see text.

changes. It is suggested that the initial effect of the mechanical stimulation is to force milk from the compressed area of mammary parenchyma into the ducts and alveoli of the rest of the gland. Thus, when pressure is removed from the compressed area and the compressed alveoli relax, the total volume of the gland will temporarily revert to a value greater than that which is seen in the resting state, and the pressure drops to a lower level. This lasts for only a short time because of the rise in pressure caused by contraction of the myoepithelial cells resulting from mechanical stimulation. The innervation of the rat mammary gland does not seem to be involved in the response to mechanical stimulation since both negative and positive phases occurred when the mammary glands were either peripherally denervated or filled with lignocaine instead of milk.

Mechanical stimulation of the mammary gland appears to cause not only myoepithelial cell contraction(1-5) but also, by physical compression of alveoli, to stretch the walls of other parts of the gland. One effect of this stretching would be to increase the diameter of the ducts, a result also achieved by the action of oxytocin on the myoepithelium which is arranged longitudinally along the ducts(6). According to our hypothesis, therefore, duct dilatation and alveolar contraction follow both mechanical and oxytocin stimulation. These effects will assist in the attainment of a high rate of milk flow to the exterior.

Summary. Mechanical stimulation of the mammary glands of lactating rats and in the one rabbit tested results in an initial reduction in intramammary pressure prior to a rise in pressure of longer duration. The reduction in pressure is particularly marked when mechanical stimulation is applied during an existing rise in intramammary pressure due to a previous oxytocin or mechanical stimulation. Summation of responses did not occur when either two consecutive mechanical stimuli or an oxytocin plus a mechanical stimulus were applied; in fact the normal intramammary pressure response to oxytocin was reduced following repeated mechanical stimulation. Neither denervation nor intra-

ductal filling of the mammary gland with lignocaine affected the response pattern.

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Influence of Strontium on the Cardiotropic Actions of Rhodochlorin and Ouabain.* (32528)

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In spite of the early observation by Ringer in 1883 that strontium possesses a positive inotropic action similar to that of calcium, the cardiotropic actions of strontium have not been extensively studied by modern techniques(1-4). We have recently reported the necessity of calcium in the positive inotropic action of ouabain and gamma-methyl rhodochlorin(5). The latter is a porphyrin derivative, which was prepared from chlorophyll *a* and extracted originally from spinach in this laboratory by the method previously described (6). The present investigation concerns the effects upon the cardiotropic actions of ouabain and gamma-methyl rhodochlorin on the frog heart when the calcium in the Ringer's perfusion solution is replaced by equimolar strontium, and with the effects of these cardiotropic agents after perfusing the heart with Ca-free Ringer's solution containing EDTA-Na₂ (ethylene diaminetetraacetate disodium). Evidence is provided to suggest that the positive inotropic action of strontium is calcium dependent and the factors other than the uptake of calcium by the myocardium(7, 8), may be important for the cardiotonic action ouabain.

Methods. The isotonic contractions and electrograms of the isolated spontaneously beating frog heart (*Rana pipiens*, 8-10 cm) were recorded on a standard physiograph as previously described(5). The "normal" Ringer's perfusion solution contained: NaCl,

112.0 mM; KCl, 1.88 mM; CaCl₂, 1.08 mM; NaHCO₃, 2.38 mM (Ca-Ringer's solution). The pH of the solution was approximately 8 with or without CaCl₂, and also with the replacement of CaCl₂ with equimolar SrCl₂ (Sr-Ringer's solution). The frog heart is relatively insensitive to changes in pH and osmolarity of the perfusion solution(5). All experiments were performed at 25 ± 1°C. Because the heart rate varied from one preparation to another, the number of beats required for the onset of maximal positive inotropic effect after a change in perfusion solution was used to quantitate differences in response(5). At least 8 experiments were performed for each of the observations.

Results. When the calcium in the Ringer's solution was suddenly replaced by equimolar strontium, the force of contractions rapidly diminished to unrecordable levels and after 2 hours perfusion with the Sr-Ringer's solution contractility did not return upon the restoration of calcium to the perfusion solution (Fig. 3-1). However, if ouabain (6.7×10^{-7} M) were present in the Sr-Ringer's solution, a positive inotropic action was observed (Fig. 1), and this cardiotonic action was related to the concentration of strontium in the perfusion solution (Fig. 2). Gamma-methyl rhodochlorin (1.7×10^{-6} M), like ouabain, produced a positive inotropic action upon hypodynamic frog heart deficient in calcium(5), but, unlike ouabain, it was without effect when present in the Sr-Ringer's solution (Fig. 4-3). When additional strontium (0.54-1.08 mM SrCl₂) was

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