mental dormancy. In studies with mammary explants from mice in midpregnancy, which are in an active phase of development (*20*), we showed that maximal accumulation of α-lactalbumin occurs in the presence of exogenous insulin and prolactin and that cortisol ($> 10^{-7}M$) exerts only inhibitory effects. However, casein synthesis in those cultured explants required the presence of insulin, prolactin, and cortisol, which was effective at $3 \times 10^{-8}M$, as shown earlier by Stockdale *et al.* (*7*). Thus it is conceivable that the response of mammary explants to hormones may be influenced by the developmental state of tissue at the time of explantation as well as by the presence of endogenous hormones that may be carried over into the explants.

The observed differential effect of cortisol suggests that the action of cortisol may be mediated by different mechanisms with respect to the accumulation of casein and α-lactalbumin. The difference may be expressed at the intercellular level and may involve the response of two types of cell, one participating in casein synthesis and the other in α-lactalbumin formation. Alternatively, the difference may be at the intracellular level, involving hormone-receptor interaction, transcriptional or translational control, or even posttranslational events. In many tissues, glucocorticoid has been shown to exert catabolic action, resulting in decreased synthesis of protein and nucleic acids and also inhibition of amino acid and glucose uptake (*21*). On the other hand, it has been shown (*22*) that high doses of cortisol retard the release of plasminogen activator, thus decreasing the concentration of the protease plasmin in mammary tissue. These actions of cortisol need to be evaluated in elucidating the differential effects of cortisol on the production of casein and α-lactalbumin in mammary gland.

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Intensity Fluctuation Spectroscopy Monitors Contractile Activation in "Resting" Cardiac Muscle

Abstract. *Intensity fluctuations in a laser beam scattered by nonbeating isolated rat cardiac muscle varied directly with the calcium concentration in the bathing fluid. The steady-state level of these fluctuations varied directly with calcium-dependent force suggesting that the intensity fluctuations reflect an interaction of calcium ions with the myofilaments. The demonstration that both a portion of resting force and the frequency of intensity fluctuations vary directly with calcium even in quiescent conditions indicates that some contractile activation is present in the resting muscle.*

A laser beam passed through muscle is scattered by structures within the muscle (*1*, *2*), and if these are either moving or changing polarizability in time, fluctuations in the intensity of the scattered beam are observed. The marked increase in the frequency of fluctuations during a tetanus and their decay to nonmeasurable levels in resting skeletal muscle (*1*, *2*) suggest that the fluctua-

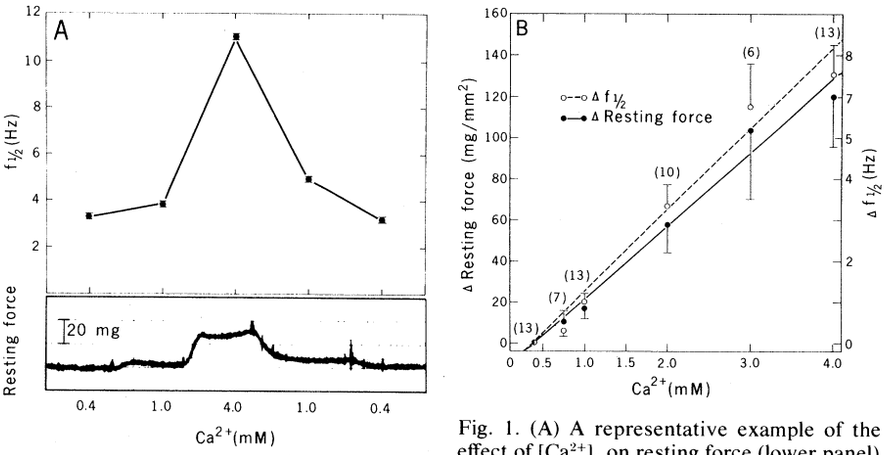

Fig. 1. (A) A representative example of the effect of $[Ca^{2+}]_e$ on resting force (lower panel) and $f_{1/2}$ (upper panel) in nonbeating cardiac muscle with an intact sarcolemma. The points represent the mean ± standard error of five determinations of $f_{1/2}$ in the steady state. In control experiments, in a given $[Ca^{2+}]_e$, steady-state measurements of $f_{1/2}$ in 12 to 16 locations across the muscle varied from one another by 10 percent or less. In other control experiments in which sucrose was substituted for a change in $[Ca^{2+}]_e$, no change in $f_{1/2}$ was observed. In this example the change in resting force in $[Ca^{2+}]_e$ from 0.4 to 4.0 mM as depicted in the actual record was 30 mg, and the total resting force was 150 mg in a $[Ca^{2+}]_e$ of 4.0 mM. The muscle cross-sectional area was 0.12 mm². (B) The change in resting force and $f_{1/2}$ as $[Ca^{2+}]_e$ is varied from 0.4 to 4.0 mM; ΔRF and $\Delta f_{1/2}$ represent the difference between the value measured in a given $[Ca^{2+}]_e$ and that in a $[Ca^{2+}]_e$ of 0.4 mM. Symbols represent the mean ± standard error of N (in parentheses) muscles at each $[Ca^{2+}]_e$. The lines in the figure were constructed from the regression analysis of ΔRF or $\Delta f_{1/2}$ and $[Ca^{2+}]_e$: $\Delta RF = [36.1\ Ca^{2+} - 15.54]$, $r = .99$, $P < .001$; $\Delta f_{1/2} = [1.98\ Ca^{2+} - 0.83]$, $r = .99$, $P < .001$. In $[Ca^{2+}]_e$ of 0.4 mM the resting force was 713 ± 62 mg/mm² and $f_{1/2}$ was 3.38 ± 0.5 Hz. Muscle cross-sectional areas averaged 0.27 ± 0.03 mm².

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tions occur when the contractile proteins are in an activated state. In cardiac muscle isolated from the rat, in contrast to skeletal muscle, we have observed intensity fluctuations even in the quiescent nonstimulated state; these fluctuations produce a speckling effect that is visible to the naked eye (*3*). Furthermore, since cardiac as opposed to skeletal muscle exhibits resting tone or force at lengths at or below the length where force development on excitation is optimal, we hypothesized that some residual contractile activation is present in cardiac muscle even in the resting or quiescent state, and that evidence for this would be fluctuations in intensity.

Although the basis of generation of the intensity fluctuations remains to be determined, evidence of a tight coupling between calcium activation of the myofilaments and the frequency of intensity fluctuations would implicate the Ca^{2+}-myofilament interaction as the initiating event for the production of the intensity fluctuations. We therefore determined the relation between the frequency of the fluctuations in intensity and resting force at a given length in nonbeating quiescent cardiac muscle bathed in differing concentrations of Ca^{2+}.

Right ventricular papillary muscles were mounted horizontally in a 3-ml chamber which facilitated measurements of force and light scattering (*4*). All studies were done at L_{max}, that length at which force development after excitation was maximal. The frequency of the intensity fluctuations $f_{1/2}$ was derived from the half-decay time of the autocorrelation function (*5*) of the laser beam scattered at an angle of 30° from the incident beam in the plane containing the long axis of the muscle.

Figure 1A shows the response of resting force and $f_{1/2}$ to step increases and decreases in the Ca^{2+} concentration of the bathing fluid, $[Ca^{2+}]_e$, over a range commonly used in studies of excitation-contraction in this species (*6*). Resting force, measured at high gain (lower panel), varied directly with $[Ca^{2+}]_e$, and in this example the Ca^{2+}-dependent portion was 20 percent of total resting force. Similarly, $f_{1/2}$ (upper panel) varied directly with $[Ca^{2+}]_e$ and the change in $f_{1/2}$ paralleled that in force. The average change in resting force and $f_{1/2}$ in 13 muscles over this tenfold range of $[Ca^{2+}]_e$ is shown in Fig. 1B. Both resting force and intensity fluctuations varied linearly and were highly correlated (r = .99) with $[Ca^{2+}]_e$ over this range, and a tight coupling was observed between the two as each varied with $[Ca^{2+}]_e$. That Ca^{2+} dependence of both the intensity fluctuations and resting force can be demonstrated in nonbeating "resting" cardiac muscle indicates that this type of cardiac muscle is neither totally resting nor quiescent, and that resting force at this length is not totally passive but is, in part, active in nature (*7*). This observation links several other phenomena observed previously in resting cardiac muscle, such as the suggestion of active cross-bridge formation in x-ray diffraction patterns (*8*) and the Ca^{2+}-dependent response to stretch (*9*), and may account in part for the oxygen consumption and heat production in the "resting" state (*10*). If one assumes that (i) Ca^{2+} activates the contractile proteins, and (ii) a Ca^{2+}-dependent change in force indicates a change in activation, both of which have a firm experimental basis (*11*), the tight coupling of Ca^{2+}-dependent force and $f_{1/2}$ strongly suggests that $f_{1/2}$ must reflect the level of Ca^{2+} activation of the myofilaments.

Although resting force and $f_{1/2}$ vary directly, the relation need not be causal; indeed, both may be independent consequences of a change of $[Ca^{2+}]_e$. We thus attempted to dissociate the two phenomena by maintaining constant $[Ca^{2+}]_e$ but altering resting force. This was accomplished by ouabain, which in high concentrations increases myoplasmic Ca^{2+} and resting force at a given $[Ca^{2+}]_e$ (*12*). Both steady-state resting (contracture) force and $f_{1/2}$ showed graded increases as ouabain concentrations increased from 10^{-6} to $10^{-3}M$ (Fig. 2A). Since $[Ca^{2+}]_e$ was constant in these experiments the dependence of $f_{1/2}$ on $[Ca^{2+}]_e$ (Fig. 1) cannot be attributed to a direct effect of extracellular Ca^{2+} but must result from changes in myoplasmic Ca^{2+} that follow changes in $[Ca^{2+}]_e$ (*13*). When the muscle was then perfused with Ca^{2+}-free medium to which EGTA was added, the intensity fluctuations were abolished and resting force was reduced to less than control. This resting force, in the absence of Ca^{2+}, probably represents the best estimate of the truly passive component of the resting force in isolated rat cardiac muscle.

In other experiments papillary muscles were also chemically "skinned" to eliminate the sarcolemmal barrier for ionic diffusion which acts to limit intracellular $[Ca^{2+}]$ (*14, 15*). As shown in Fig. 2B, $f_{1/2}$ was essentially zero (indicated by a flat autocorrelation function) when the perfusate lacked calcium. Increases in

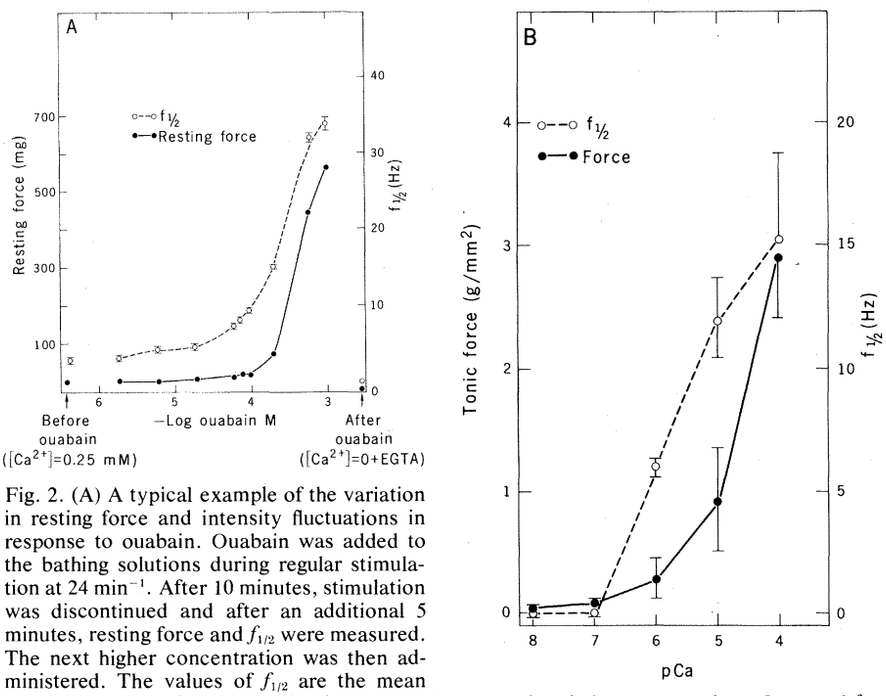

Fig. 2. (A) A typical example of the variation in resting force and intensity fluctuations in response to ouabain. Ouabain was added to the bathing solutions during regular stimulation at 24 min^{-1}. After 10 minutes, stimulation was discontinued and after an additional 5 minutes, resting force and $f_{1/2}$ were measured. The next higher concentration was then administered. The values of $f_{1/2}$ are the mean ± standard error of five determinations. Over the range of ouabain concentrations, force and $f_{1/2}$ were well correlated (r = .86, $P < .001$). Similar results were observed in several additional muscles. Muscle cross-sectional area was 0.28 mm^2. (B) Ca^{2+}-dependent force and $f_{1/2}$ in chemically "skinned" muscles. The composition of the "skinning" solution was as in (*14*), and of the relaxing and contracture solutions, as in (*15*). The symbols represent the mean ± standard error of the steady-state levels of tonic force and $f_{1/2}$ in three muscles. Tonic or Ca^{2+}-dependent force is the difference between the force at any given pCa and the force in the relaxing solution, in the absence of Ca^{2+} and in the presence of EGTA. Over the range of pCa in the figure, the increase in $f_{1/2}$ paralleled that in contracture force (r = .86, $P < .02$).

$[Ca^{2+}]_e$, however, resulted in step increases in $f_{1/2}$ concurrent with increases in force, thus further substantiating the link between Ca^{2+}-dependent force and the intensity fluctuations.

We interpret our results to indicate that the frequency of intensity fluctuations reflects the level of Ca^{2+} activation of the contractile proteins. This suggests that as a result of Ca^{2+} activation the myofilaments are either set in motion or are subject to changes in their refractive indices. However, the magnitude of the fluctuations must not be interpreted as indicative of the precise dynamics of the myofilaments, for although the Ca^{2+}-dependent fluctuations probably originate in myofilaments within the sarcomere, motion between sarcomeres within a given fiber or between the many myofibers composing the muscle probably contributes, to an undetermined extent, to the measurement of the intensity fluctuations. In spite of this potential limitation, measurements of intensity fluctuations adds a new dimension to the study of nonbeating cardiac muscle, permitting the monitoring of the relative level of activation in the nonbeating state and its coupling to subsequent excitation-contraction in cardiac muscle.

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5. The beam of a helium-neon laser (Spectra 147P, λ = 632.8 nm) was focused onto papillary muscle suspended in the chamber. Light scattered by the muscle was collimated by a double pinhole system and detected by a photomultiplier tube (Hamamatsu R928). The photocurrent fluctuations were analyzed by an a-c coupled autocorrelator (Honeywell Saicor 42A).
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7. In the muscles in Fig. 1B, total resting force, *RF*, in $[Ca^{2+}]_e$ of 4.0 m*M* averaged 826 ± 76 mg/mm^2. The ΔRF, or Ca^{2+}-dependent portion of *RF* averaged 121 ± 25 mg. Thus at least 15 percent of resting force at L_{max} in $[Ca^{2+}]_e$ of 4.0 m*M* in these preparations is Ca^{2+}-dependent. This is a low estimate of the Ca^{2+}-dependent portion of resting force since reducing the $[Ca^{2+}]_e$, from 0.4 m*M* to zero results in a further reduction of *RF*.
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Control of the Rhesus Monkey Menstrual Cycle: Permissive Role of Hypothalamic Gonadotropin-Releasing Hormone

Abstract. *In rhesus monkeys with hypothalamic lesions (which appear to abolish the endogenous production of gonadotropin-releasing hormone), normal ovulatory menstrual cycles were reestablished by an unvarying, long-term replacement regimen consisting of one intravenous pulse of synthetic gonadotropic-releasing hormone per hour. This finding is in accord with the hypothesis that the pattern of pituitary gonadotropin secretion throughout the menstrual cycle (basal secretion interrupted, once every 28 days on the average, by a preovulatory surge) is not directed by alterations in hypothalamic gonadotropic-releasing hormone secretion but by the ebb and flow of ovarian estrogens acting directly on the pituitary gland.*

In women and rhesus monkeys, the abrupt discharge of the pituitary gonadotropic hormones that culminates in ovulation seems to be initiated by the rising tide of circulating estradiol secreted by the rapidly developing Graafian follicle near midcycle (*1*). Evidence has recently been advanced in favor of the hypothesis that, in the rhesus monkey, this so-called positive feedback action of estradiol is at the level of the pituitary gland rather than that of the nervous system (*2*, *3*) and that hypothalamic gonadotropic-releasing hormone (GnRH) may, therefore, play only a permissive, albeit obligatory, role in this regard (*4*). We tested and affirmed this hypothesis, which predicts that, in rhesus monkeys devoid of endogenous GnRH, normal ovulatory 28-day menstrual cycles should be subserved by an unvarying GnRH replacement regimen.

In order to abolish endogenous GnRH production, bilateral radio-frequency lesions were placed in the arcuate region of the medial basal hypothalamus of seven adult, intact female rhesus monkeys (*5*). The functional completeness of the lesions was established by the reduction in plasma luteinizing hormone (LH) and follicle-stimulating hormone (FSH) to unmeasurable concentrations and by the loss of responsiveness to the positive feedback action of estradiol when estradiol benzoate (EB) was subcutaneously injected 1 and 3 weeks after the operation (Fig. 1).

The animals were then placed on a GnRH replacement regimen previously found to reestablish gonadotropin secretion in ovariectomized monkeys with similar hypothalamic lesions (*2*, *6*). The GnRH was infused intermittently at a rate of 1 μg/min for 6 minutes once every hour, for 65 to 158 days, through permanently implanted cardiac catheters connected to Harvard infusion pumps by swivel joints; these devices permitted continuous access to the venous circulation without restraining the animals (*2*).

After the GnRH replacement regimen ended, the effectiveness of the hypothalamic lesions was reverified by the continued undetectability of the gonadotropic hormones in plasma and by the unresponsiveness to EB administration, even after bilateral ovariectomy. The time courses of LH, FSH, the ovarian hormones, and prolactin in plasma were assessed by specific radioimmunoassays (*7*).

Within 2 to 3 days after the initiation of the pulsatile GnRH replacement regimen, plasma gonadotropin concentrations began to rise, and, in most animals, estradiol appeared in the circulation shortly thereafter. In four of the seven monkeys in this series (Fig. 1), estradiol then rose rapidly and initiated gonadotropin surges. These surges in turn resulted in ovulation and normal corpus luteum formation, as judged by the resulting time courses in plasma progesterone concentration, which resembled those seen in normal, spontaneous menstrual cycles. When more than one ovulatory menstrual cycle was observed in the course of GnRH administration, the intervals between LH peaks were 28 to 33 days—within the normal range of our rhesus monkey colony.

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