initially, a gradual increase in beat frequency without a change in bend angle, corresponding to a gradual increase in sliding velocity (*7*). During the later stages of digestion by trypsin, there were often larger increases in frequency, accompanied by decreases in bend angle.

The effect of digestion by elastase is exactly that expected if the interdoublet linkages are involved in regulating the amplitude of flagellar bending and if these elastic interdoublet linkages are particularly sensitive to digestion by elastase. Elastase appears to have a much more specific effect than trypsin, and may be a better choice for future studies of sliding disintegration following enzymatic degradation of flagella and cilia.

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8. The reactivation solutions for these experiments contained 0.15*M* KCl, 20 m*M* tris buffer, 2.0 m*M* EGTA, 2 m*M* dithiothreitol, 2 percent polyethylene glycol, and $MgSO_4$, adenosine triphosphate (ATP), and $CaCl_2$ to give 0.20 m*M* $MgATP^{2-}$, 1.0 m*M* Mg^{2+}, and 10^{-9}*M* Ca^{2+}, at *p*H 8.2. All incubations and measurements were made at 18°C.
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α-Lactalbumin–Casein Induction in Virgin Mouse Mammary Explants: Dose-Dependent Differential Action of Cortisol

Abstract. *The interplay of insulin, cortisol, and prolactin induces synthesis of casein and α-lactalbumin in cultured mammary explants from mature virgin mice. A striking difference has been found between the optimal concentrations of cortisol required for maximal induction of the two milk proteins in vitro: 3×10^{-8} molar for α-lactalbumin and 3×10^{-6} molar for casein. Moreover, 10^{-7} to 10^{-5} molar cortisol caused progressive inhibition of α-lactalbumin accumulation. Such differential actions of cortisol may partly account for the asynchronous synthesis of the two proteins during pregnancy.*

Casein and α-lactalbumin are the major milk proteins synthesized by lactating mammary gland. Casein is a group of phosphoproteins that comprise more than 50 percent of milk protein (*1*); α-lactalbumin is a whey protein that can modify the substrate specificity of galactosyltransferase to include glucose, thus enhancing the synthesis of lactose (*2*).

Juergens *et al.* (*3*) showed that when mammary explants from mice in midpregnancy are cultured in a chemically defined medium containing insulin, cortisol, and prolactin, each at a concentration of 5 μg/ml, there is marked stimulation of the incorporation of inorganic phosphate into the Ca^{2+}-rennin-precipitable proteins, a large portion attributable to casein. Subsequently, Turkington *et al.* (*4*), using the same culture conditions, reported that the triple hormone combination stimulates mammary explants to make α-lactalbumin, which was assayed by the lactose synthetase system. These initial findings have been confirmed by others and these methods of measuring casein synthesis and α-lactalbumin have been widely employed (*5*-*9*).

During our investigation of the role of cortisol in the development of mammary gland in vitro, it became evident that the previous assay procedures for casein and α-lactalbumin were not satisfactory from the standpoint of accurate quantitative measurement. We have therefore used an immunochemical procedure to determine casein synthesis and a modified quantitative assay method to measure the amount of α-lactalbumin, utilizing the lactose synthetase system with pure mouse α-lactalbumin as a standard. With these methods, we have examined the dose-response relation between cortisol and the induction of either casein or α-lactalbumin. To our knowledge, this is the first time such comparative studies have been done in a systematic way. The data presented in this report reveal a striking difference between the concentrations of cortisol needed for maximal induction of casein synthesis and α-lactalbumin accumulation.

Mammary explants from 3- to 4-month-old virgin female C3H/HeN mice were cultured in a chemically defined synthetic culture medium, medium 199, as described previously (*10*). Casein synthesis was determined by incubating explants in the presence of a ^{3}H-labeled amino acid mixture (10 μCi/ml) for 4 days under the indicated conditions and subsequently immunoprecipitating isotopically labeled casein with mouse casein antibody prepared in rabbits (*11*). The amount of α-lactalbumin in mammary explants was determined as the activity of α-lactalbumin in the lactose synthetase reaction, using the modified method of Fitzgerald *et al.* (*12*) with pure mouse α-lactalbumin (*13*) as a standard.

Figure 1 shows the effect of varying the concentration of cortisol on the accumulation of α-lactalbumin, casein, and total protein synthesized in mammary explants from mature virgin mice in culture. During a 4-day culture period, accumulation of casein was low in the culture system with insulin or insulin plus prolactin. However, addition of increasing amounts of cortisol to the medium containing insulin and prolactin effected dose-dependent increases in casein. Maximal accumulation was effected by about 3×10^{-6}*M* cortisol. Addition of cortisol alone or cortisol combined with insulin or prolactin did not cause an increase in casein synthesis.

A different dose-response relation was observed in the accumulation of α-lactalbumin. The level of α-lactalbumin was low in the culture system with insulin, but was elevated 2.5-fold by the addition of prolactin with insulin. Addition of up to 3.0×10^{-8}*M* cortisol with insulin and prolactin caused further increases in α-lactalbumin, and the amount of the milk protein was twice that in the culture with insulin and prolactin. However, at higher concentrations ($> 10^{-7}$*M*), cortisol had an inhibitory effect. Addition of 3×10^{-6}*M* cortisol, an optimal concentration for casein synthesis, reduced the tissue content of α-lactalbumin more than 5-fold in the insulin-prolactin system.

The stimulatory effect of cortisol on total protein synthesis is not apparent up to 3×10^{-8}*M* but is manifested at higher concentrations. This can be explained as follows. About 50 percent of the total protein synthesized in maximally stimulated tissue explants is casein; less than 0.2 percent is α-lactalbumin, as determined by immunochemical analysis with antibody to mouse α-lactalbumin (*13*). In addition, considerable amounts of noncasein proteins are synthesized by mammary explants cultured with insulin alone (*14*), which results in higher baselines for total protein synthesis.

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In separate experiments (data not shown), other glucocorticoids, including dexamethasone, deoxycorticosterone, cortisone, and prednisolone, were also found to have a stimulatory effect on α-lactalbumin accumulation at lower concentrations (~ 10^{-9} to $10^{-8}M$) and an inhibitory effect at higher concentrations (~ 10^{-7} to $10^{-5}M$). On the other hand, similar studies with insulin and prolactin indicated that the two polypeptide hormones do not have such dose-dependent opposing effects on milk protein synthesis.

Table 1 shows the effects of various concentrations of cortisol on the rate of casein synthesis, as determined on day 3 of culture, when casein synthesis was at a peak (*3*). The maximal rate of casein synthesis was observed in the presence of insulin, prolactin, and $3 \times 10^{-6}M$ cortisol. These results are in accord with the data in Fig. 1, indicating that the stimulatory effect of cortisol on the accumulation of casein is largely due to enhancement of casein synthesis. Similar experiments to determine the rate of α-lactalbumin synthesis were carried out with mouse α-lactalbumin antibody. These experiments proved difficult, mainly because the amount of α-lactalbumin in cultured explants was so small (< 0.2 percent)—often below the control value in immunoprecipitation reactions. Preliminary analysis of immunoprecipitates by sodium dodecyl sulfate-polyacrylamide gel electrophoresis indicated, however, that cortisol has a dose-dependent differential effect on α-lactalbumin synthesis.

Table 1. Effect of cortisol concentration on rate of casein synthesis in cultured mammary explants from virgin mice.

Culture condition	Casein synthesis* (count/min-mg in 4 hours)
Insulin	Undetectable
Insulin and prolactin	10
Insulin, prolactin, and cortisol	
$3 \times 10^{-8}M$	400
$3 \times 10^{-7}M$	800
$3 \times 10^{-6}M$	1200
$8 \times 10^{-6}M$	1180

*The rate of casein synthesis in cultured explants is expressed as counts per minute per milligram of tissue in 4 hours. It was determined by pulse-labeling with ^{3}H-labeled amino acid mixture (60 μCi per milliliter of medium) during 68 to 72 hours of culture. Other details are given in the legend to Fig. 1. Each value is the average of triplicate determinations; the standard error was less than 7 percent.

The present findings—that the optimal concentration of cortisol ($3 \times 10^{-6}M$) for casein synthesis is about 100 times higher than that for α-lactalbumin and, moreover, is inhibitory to the accumulation of α-lactalbumin in mammary gland—are of interest for several reasons.

During the second half of pregnancy, casein synthesis in mammary gland rises progressively, whereas the α-lactalbumin level remains low, rising rapidly to a maximum after parturition (*15*). It was suggested that the differential kinetics of the two proteins during pregnancy is affected by progesterone, because progesterone selectively inhibits the increase in α-lactalbumin in vitro (*16*) and because the concentration of progesterone in the plasma changes from high levels during days 4 to 18 of pregnancy to low levels just before parturition (*17*), which correlates with the pattern of changes in α-lactalbumin. Our results indicate, however, that the concentration of glucocorticoid used in the previous study (*16*), 5 μg/ml, is inhibitory to α-lactalbumin accumulation. This raises the question of whether the observed inhibition of α-lactalbumin by progesterone represents a genuine selective action of progesterone or whether cortisol can also contribute to maintaining the low level of α-lactalbumin in vivo as well as in vitro. It has been shown that the concentration of glucocorticoid in the plasma rises progressively during the second half of pregnancy up to around $10^{-6}M$ and falls to $10^{-8}M$ after parturition (*18*). These high and low levels of cortisol in the plasma correspond to the optimal concentrations for casein and α-lactalbumin accumulation, respectively, as determined in the present studies. Thus, these data taken together suggest that asynchrony of synthesis of the two milk proteins during pregnancy may, at least in part, be effected by the change in the level of glucocorticoid in the plasma.

Organ culture of mouse mammary gland has been widely used to study the hormonal regulation of the functional differentiation of mammary epithelium. In most instances, both casein and α-lactalbumin have been used as specific markers of differentiated function of mammary cells in a culture system containing insulin, prolactin, and high concentrations of glucocorticoid (~ 10^{-6} to $10^{-5}M$). In this culture system, numerous agents including other hormones have been examined for their effects on milk protein synthesis. In view of the findings reported here, however, the results of previous studies, particularly those related to α-lactalbumin (*19*), may need to be reexamined because of inadequacies in the culture conditions or the assay method.

The present experiments were carried out with mammary explants from virgin mice, which are in a state of develop-

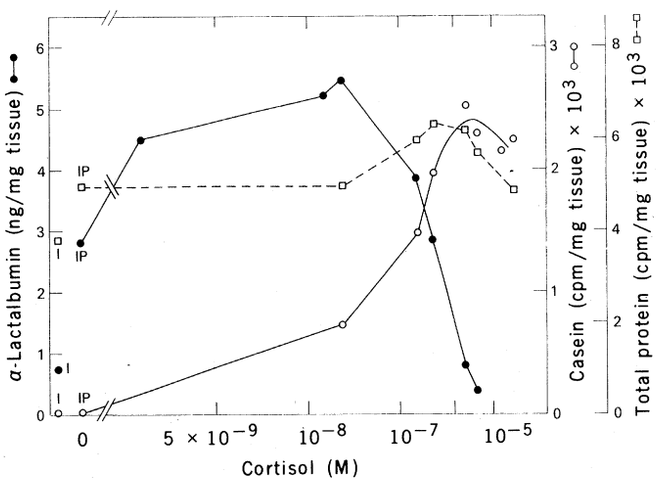

Fig. 1. Effect of cortisol concentration on (○) casein synthesis, (●) α-lactalbumin content, and (□) total protein synthesis in mammary explants in culture. Mammary explants from C3H/HeN mature virgin mice were cultured in medium containing combinations of insulin (*I*; 5 μg/ml), prolactin (*P*; 5 μg/ml), and various concentrations of cortisol (*10*). Bovine prolactin that had been freed of contaminating vasopressin and oxytocin (*23*) was used. The concentrations of the two polypeptide hormones were optimal for synthesis of casein and α-lactalbumin in vitro (*4*, *20*). Casein synthesis and the amount of α-lactalbumin in mammary explants were measured as described in the text. Total protein synthesized by tissue explants was determined by the incorporation of ^{3}H-labeled amino acid into trichloroacetic acid–insoluble materials. The assay mixture for the activity of α-lactalbumin consisted of 50 μl of a solution containing 27 m*M* tris-HCl (*p*H 7.4), 6.8 m*M* $MnCl_2$, 1.4 m*M* uridine diphosphate (UDP) galactose supplemented with ^{14}C-labeled UDP galactose (30,000 count/min), 1 m*M* guanosine triphosphate (GTP), and 40 m*M* glucose; 10 μl of an excess amount of bovine galactosyltransferase (2.0 U/ml) in 20 m*M* tris-HCl (*p*H 7.4) containing 10 m*M* $MgCl_2$ and 100 m*M* KCl; and 40 μl of test solution. Test solution was prepared by homogenizing tissue explants with approximately five volumes of 20 m*M* tris-HCl (*p*H 7.4) containing 10 m*M* $MgCl_2$, 100 m*M* KCl, and 2 percent Triton X-100. After standing for 30 minutes in an ice bath, the homogenate was centrifuged at 2000 rev/min for 20 minutes and the supernatant was used for the assay. Parallel assays were carried out without glucose or transferase or GTP to correct for the background radioactivity contributed by hydrolase, endogenous acceptor, endogenous transferase, or GTP. Each point represents an average of closely agreeing duplicate determinations. The data represents one of several similar experiments that gave essentially the same results.

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-

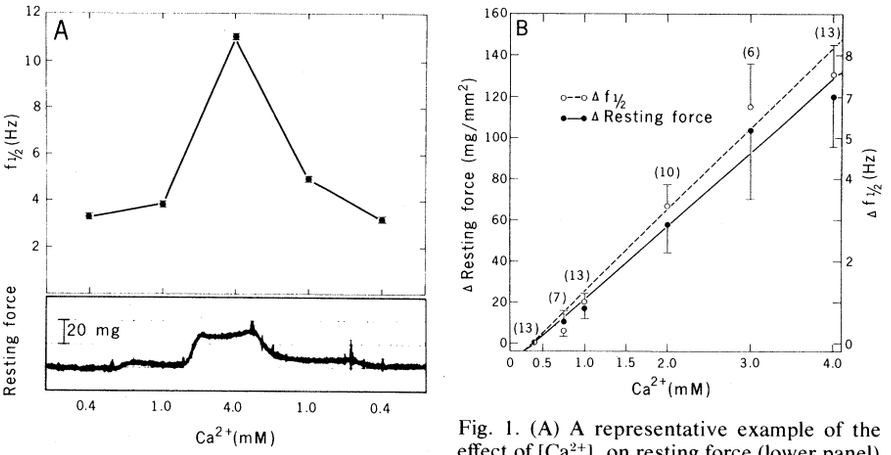

Fig. 1. (A) A representative example of the effect of $[Ca^{2+}]_e$ on resting force (lower panel) and $f_{1/2}$ (upper panel) in nonbeating cardiac muscle with an intact sarcolemma. The points represent the mean ± standard error of five determinations of $f_{1/2}$ in the steady state. In control experiments, 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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