lower concentration of cyclic GMP (0.14 μM) than those (histone, 400 μg/ml; cyclic GMP, 100 μM) previously reported to have had the same effect[3,4]. The weakness of the modulator-dependent protein kinase II peak indicates the possible loss of enzyme activity during the process of preincubation and dissociation. It proved to be much easier to observe the converted modulator-dependent protein kinase II by using a cyclic GMP-dependent protein kinase-rich tissue, such as lung in this study, as the starting sample for preincubation. When non-cyclic GMP-dependent protein kinase-rich tissue, such as testis, was tried, no modulator-dependent protein kinase II activity was detected[14]. The successful separation between greatly phosphorylated protein substrates, such as protamine, and slightly phosphorylated stimulatory protein kinase modulator, strongly suggests that stimulatory protein kinase modulator was a real stimulatory factor, and not a substrate[14]. With the in vitro verification of 2 forms of modulator-dependent protein kinases, future investigations may study the conditions needed to convert cyclic GMP-dependent protein kinase holoenzyme into its subunit and to reverse the process in vivo, and the possible existence of multiple forms of modulator-dependent protein kinases in vivo and their physiological functions.

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Progesterone stimulates energy-dependent contraction of swollen heart mitochondria[1]

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Summary. Progesterone stimulates the rate and efficiency of respiration-dependent contraction of heart mitochondria in nitrate salts at alkaline pH. Ion extrusion under these conditions is normally slow and inefficient due to the elevated permeability of the membrane to monovalent cations through a putative uniport pathway. Progesterone also inhibits passive swelling under these conditions and appears to act by restricting cation influx through the uniport pathway.

Progesterone has been shown to affect the respiration and the permeability properties of mitochondira[2-7]. At low concentrations (approximately 50 μM) progesterone inhibits NADH oxidation at a site between flavoprotein and coenzyme Q[2-4] while higher concentrations (150 μM) inhibit ADP-stimulated succinate oxidation in an uncoupler-sensitive manner[3-5]. At concentrations above 200 μM progesterone acts as a weak uncoupler and the detergent-like properties of the steroid contribute to swelling of mitochondria and the loss of matrix components[6,7].

The energy-dependent contraction of swollen mitochondria appears to be an osmotic response to the electroneutral extrusion of cations on an endogenous cation/H^+ exchanger[8-11]. At neutral pH this extrusion of cations is a rapid and efficient reaction[10], but at pH 8.3 the influx of cations through a putative uniport pathway (alkaline uniport) appears to undermine efficiency and reduce the rate of net cation extrusion and contraction[10,11]. This paper reports that progesterone and other steroids inhibit passive swelling and stimulate respiration-dependent contraction of heart mitochondria at alkaline pH.

Materials and methods. The preparation of beef heart mitochondria by a Nagarse method and the simultaneous monitoring of respiration, pH, and swelling and contraction by changes in absorbance at 540 nm were carried out as previously described[10,12].

Results and discussion. Heart mitochondria swell spontaneously when suspended in nitrate salts at pH 8.3 and 37 °C in the absence of metabolic energy[9-11] (figure 1). Swelling under these conditions represents an osmotic response to the passive influx of nitrate and monovalent cation. Swollen mitochondria extrude accumulated ions and contract when respiration is initiated. Cation extrusion appears to occur via an endogenous cation/H^+ exchanger which is dependent on the ΔpH component of the protonmotive force[9-11]. Progesterone at 160 μM inhibits spontaneous swelling and activates succinate-supported contraction at pH 8.3 (figure 1). This activation of contraction is seen whether progesterone is added initially or immediately before succinate. Respiratory control, much like that associated with contraction at neutral pH[10] is also induced by progesterone under these conditions. The elevated respiration accompanying contraction declines to a low (approximately state 4) rate after contraction is complete and a steady-state volume is maintained (figure 1, B). In the absence of progesterone respiration is elevated to a level approximately that of state 3 and is linear to anaerobiosis (figure 1, B).

Titration of respiration-dependent contraction in Na^+ nitrate at pH 8.3 (figure 2) shows the optimum activation of the rate of concentration is 3.8-fold and occurs at 160 μM progesterone. The rate of respiration during contraction declines with increasing progesterone concentration and at 240 μM the rate of respiration during concentration approximates the controlled rate observed at lower concentrations. The efficiency of contraction (rate of contraction/rate

of respiration) increases throughout the concentration range tested and is stimulated approximately 8-fold by 320 µM progesterone. Other steroids, notably testosterone and corticosterone, produced similar effects at higher concentrations than progesterone.

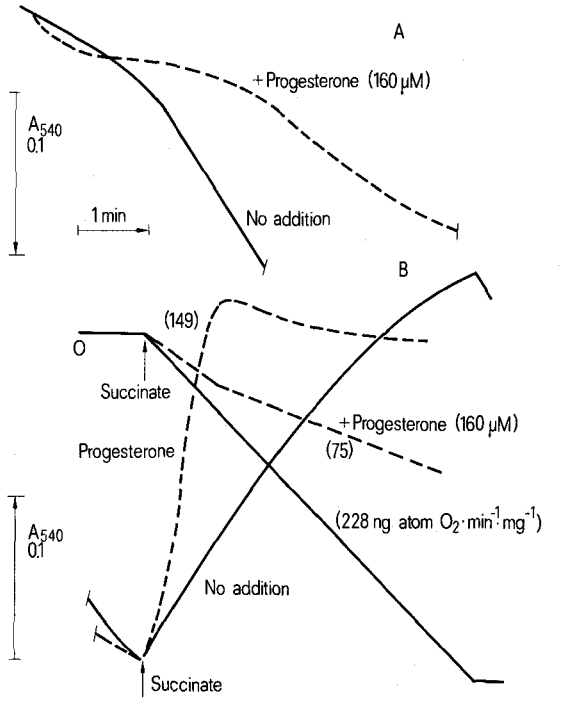

Fig. 1. Inhibition of passive swelling (A) and activation of respiration-dependent contraction (B) of heart mitochondria by progesterone in Na^+ nitrate, pH 8.3. Beef heart mitochondria (0.5 mg/ml) were suspended at 37 °C in a medium (5 ml total volume) of Na^+ nitrate (100 mM), Tris (2 mM, pH 8.3), rotenone (5 µM), and sucrose (5 mM, added with the mitochondria). Absorbance at 540 nm and oxygen content were recorded simultaneously. In A progesterone was present initially. In B contraction was initiated by Na^+ succinate (3 mM) following passive swelling of approximately 0.2 A. Progesterone was added immediately before the succinate. Respiration rates (ng atom $O_2 \cdot min^{-1} \cdot mg^{-1}$) are given in parenthesis by the oxygen traces.

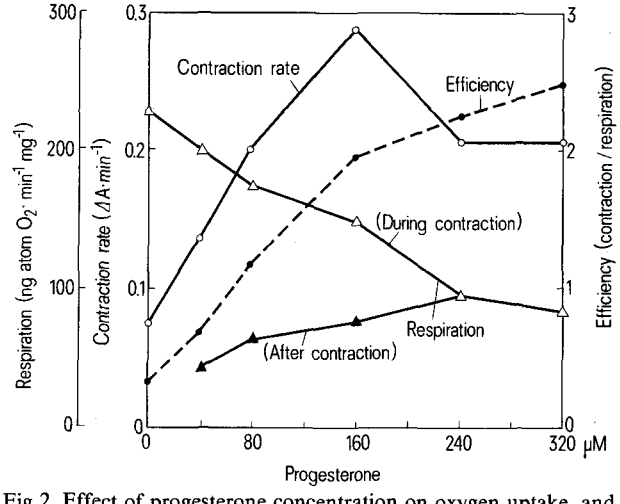

Fig. 2. Effect of progesterone concentration on oxygen uptake, and rate and efficiency of respiration-dependent contraction in Na^+ nitrate, pH 8.3. Progesterone was added immediately before succinate following 0.2 A passive swelling. Other conditions were identical to those described in fig. 1.

The activation of respiration-dependent contraction, the induction of respiratory control, and the inhibition of passive swelling (figure 1) all suggest that progesterone blocks cation influx at alkaline pH and enhances cation extrusion by decreasing influx-efflux cycling of cations. The inhibition of cation influx and stimulation of efflux by the steroid strongly support the concept that these two processes occur by separate pathways in mitochondria[9-11,13]. The concentration of progesterone effective in stimulating contraction of mitochondria is less than that reported to cause nonspecific permeability changes, swelling, and loss of pyridine nucleotides[6,7], but is approximately the same as that found to inhibit ADP-stimulated succinate oxidation in rat liver mitochondria[3-5]. It has been suggested that progesterone inhibits succinate oxidation by interfering with uptake of protons into the mitochondrion[5] and it is possible that a similar decrease in nonspecific permeability to H^+ contributes to the increase in efficiency of contraction observed in the present studies.

The similar effects of progesterone, local anesthetics[11], and dicyclohexyl-carbodiimide[13] on the contraction of swollen mitochondria suggest a common effect on membrane properties rather than interaction of these diverse reagents with specific transport components. The steroid effects described here may result simply from their ability to fit or pack into membranes and thus be secondary to their specific physiological action[14,15]. In this regard, the ability of steroids to protect membranes against mechanical and osmotic disruption is well-known[14,16]. All of these results suggest that the monovalent cation-uniport pathways represent rather nonspecific leaks which are produced by alterations in the mitochondrial membrane under given experimental conditions. In contrast, the cation/H^+ exchanger which appears to be unaffected by these reagents may involve a more specific entity. Effective inhibitors of the exchange pathway have not yet been reported and such inhibitors are clearly needed in order to clarify the precise relationship between uniport and exchange pathways for monovalent cations in mitochondria.

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