# Effects of Caffeine on the Electrical and Mechanical Activity of Guinea-Pig Ureter Smooth Muscle

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**Abstract.** The effects of caffeine on the electrical and mechanical activity of the guinea-pig ureter smooth muscle were studied. Under untreated conditions caffeine mainly showed inhibitory action on the ureter, inhibiting the evoked action potentials and phasic contractions as well as potassium contracture. Caffeine was also found to suppress the low-Na contracture of Na-loaded ureter muscle. It is established that Na-loaded tissue is able to generate transient contracture in response to caffeine application at 37 °C. These caffeine contractures could be evoked under completely removed $[Ca^{2+}]_o$ and in the presence of high doses of Ca-channel blockers (nifedipine, diltiazem, Mn ions) and could be reversibly blocked by tetracaine, procaine and benzocaine. Caffeine contractures could also be produced by the ureter muscle placed in isotonic K-solution. Cooling significantly potentiated low-Na, potassium and caffeine contractures of the ureter muscle. Filling of the store is totally dependent on the entry of Ca ions from the extracellular $Ca^{2+}$ store sites which sequester Ca ions entering the cell on either Na—Ca exchange or via voltage operated Ca channels.

**Key words:** Ureter smooth muscle — Caffeine-sensitive store — Caffeine stimulant and relaxant action

## Introduction

Caffeine has both stimulatory and relaxant effects on different smooth muscles. It often produces an initial stimulant effect that rapidly declines and is replaced by an inhibitory one (for a review, see Bolton 1979). Caffeine is believed to release calcium from sites where the latter is bound within the cell (Itoh et al. 1981; Saida 1982). Caffeine is known to release Ca from sarcoplasmic reticulum (s.r.) of skeletal (Weber 1968; Weber and Herz 1968), heart ( Chapman and Miller 1971; Jundt et al. 1975) and various smooth muscles (Kuriyama (1981). Caffeine is highly specific in its action on s.r., being without effect on the calcium turnover in

isolated mitochondria or in sarcolemmal vesicles (Weber 1968; Thorpe and Seeman 1971). The inhibitory or relaxant action of caffeine resembles that of papaverine in several ways (for a review, see Bolton 1979).

In the present study the effects of caffeine on the ureter smooth muscle were studied under various experimental conditions. Ureter smooth muscle, like cardiac muscle, generates an action potential which consists of an initial fast and a subsequent slow component (Shuba 1977), and similar to mammalian heart muscle, the ureter is able to develop tension upon Na-withdrawal when the activity of the Na—K pump has been arrested (Aickin et al. 1984). It has been shown that under standard conditions caffeine has an inhibitory action on contractions of the ureter smooth muscle induced by membrane depolarization and reversion of the Na—Ca exchanger. Caffeine could also induce transient contractures of ureter smooth muscle loaded with Na ions or placed in isotonic K solution, due to Ca release from the intracellular Ca store. Factors influencing $Ca^{2+}$ loading in the intracellular store sites in the ureter muscle were studied.

## Materials and Methods

Simultaneous electrical and mechanical records were obtained from isolated pieces of whole guinea-pig ureters using the double sucrose gap method (Bülbring and Tomita 1969). Action potentials were initiated by depolarizing rectangular current pulses of slightly suprathreshold size (in the order of $10^{-7}$ A) of short duration (20—100 ms) to avoid the possible influence of prolonged depolarization on the shape and amplitude of the action potential. Tension alone was recorded with the continuous superfusion technique described in detail by Brading and Sneddon (1980). Four tissue pieces, each about 1 cm long, could be studied simultaneously by the use of four chambers, and contractions were recorded on a 12 channel oscilloscope.

A modified Krebs solution of the following composition was used (mmol/l): $Na^+$, 120.3; $K^+$, 5.9; $Tris^+$, 16.6; $Ca^{2+}$, 2.5; $Mg^{2+}$, 1.2; $Cl^-$, 150.2; glucose, 11.5; equilibrated with 100 % $O_2$, pH 7.4. Na-free solutions were made by replacing $Na^+$ isoosmotically with $K^+$ or $Tris^+$. Na-loading of the tissue was done by exposing the muscle to ouabain ($10^{-4}$ mol/l), a K-free solution or a cold bath for 40—60 min.

The following drugs were used: caffeine, procaine, tetracaine, QX-314 (B.D.H., U.K.), nifedipine (BAY, U.K.), diltiazem (Tanabe, Japan), lidocaine (EGYT, Hungary), benzocaine (Pharmprom, USSR).

## Results

### *Caffeine and evoked electrical and mechanical activity*

Caffeine at 1 mmol/l reduced the duration of the plateau component as well as the amplitude of spikes of the action potential of the ureter muscle at 37 °C (Fig. 1). Simultaneously, the amplitude and the duration of phasic contraction was decreased. The membrane potential and membrane resistance were practically unaffected. The effect was fully revesible (Fig. 1*D*). Complete blockade of both

action potential and phasic contraction was seen with higher caffeine concentrations (5—10 mmol/l).

*Caffeine and potassium contracture*

Effect of different concentrations of caffeine on the potassium (126 mmol/l – $K^+$) contracture of the ureter muscle is illustrated in Fig. 2*A*. In *b*, *c*, *d* the tissue was pretreated with caffeine for 1 min to see the effect of caffeine on resting muscle and to minimize the possible side effect of this methylxantine; then potassium contracture was evoked in continuous presence of caffeine. Fig. 2*A* shows that caffeine did not change the resting muscle tension but dose-dependently inhibited both the phasic and the tonic component of the potassium contracture.

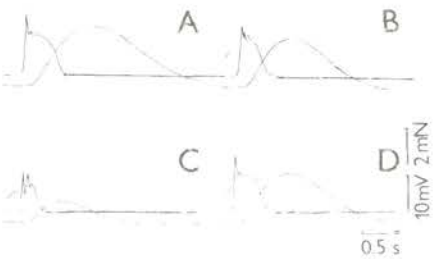


**Fig. 1.** Effect of caffeine (1 mmol/l) on the ureter smooth muscle. The upper trace shows the electrical and the lower one the mechanical response to a suprathreshold constant current pulse of short duration (double sucrose-gap method): *A*, in normal Krebs solution; *B*, *C*, during exposure to caffeine for 2 and 5 min; 5, after the washout with Krebs solution for 5 min. The stimulant current pulse is marked by changes in membrane potential preceding the action potential. The strength and duration of the pulse was altered during caffeine treatment (*C*).

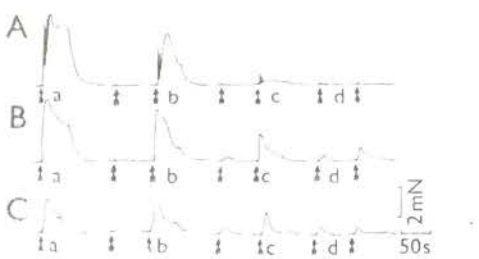


**Fig. 2.** Effects of caffeine on 126 mmol/l-K (Na-free) contractures of normal (*A*) and Na-loaded (*B*) tissue and Na-free (Tris) contracture of Na-loaded ureter (*C*). *A*, *a*, *B*, *a*, high-K contracture of normal and Na-loaded tissue in the absence of caffeine; *C*, *a*, Na-free (Tris) contracture of Na-loaded ureter in the absence of caffeine. The beginning of each trace marked by *b*, *c*, *d* shows effects of caffeine at 2, 10 and 20 mmol/l on resting muscle (first arrow) and potassium contracture of normal (*A*) and Na-loaded (*B*) tissue as well as Na-free (*C*) contracture of Na-loaded tissue (second arrow). Note the appearance of transient caffeine contractures in Na-loaded tissue (*B*, *C*) and their absence in untreated tissue (*A*). In this and subsequent figures the mechanical activity was recorded by superfusion tension recording.

The block was almost complete at 10 mmol/l of caffeine (Fig. 2*A*, *c*). It is noticeable that caffeine had no effect on resting muscle tension even in a concentration as high as 20 mmol/l (Fig. 2*A*, *d*).

*Caffeine and potassium and low-Na contracture of Na-loaded ureter*

Previously we found that Na-loaded ureter muscle responds to removal of $[Na]_o$ with a strong contracture (Aickin et al. 1984). When K ions were used as Na-substitute, the entry of Ca ions into Na-loaded tissue was promoted by both the voltage operated Ca channels and the Na-Ca exchange (Aickin et al. 1984). The Na-loaded ureter muscle responded to application of caffeine at 37 °C with a transient contracture (Fig. 2*B*, *c*). The potassium and low-Na contracture of the Na-loaded tissue showed a lower sensitivity to caffeine than potassium contracture of untreated tissue (Fig. 2*A*, *B*, *C*). However, the block was incomplete even at a concentration of caffeine as high as 20 mmol/l (Fig. 2*B*, *d*, *C*, *d*). Application of caffeine during the development of low-Na contracture normally caused transient contracture, with an amplitude higher than that observed in the Na-loaded resting muscle (Fig. 3). The amplitude of the caffeine contracture was always higher when K ions were used as Na substitute (Fig. 4).

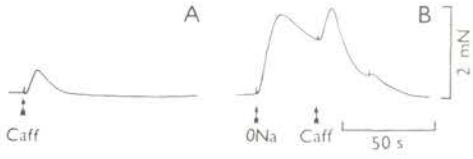


**Fig. 3.** Effect of caffeine (20 mmol/l) on Na-loaded ureter at rest (*A*) and during the development of Na-free contracture at 37 °C (*B*). Note the increase in amplitude of caffeine contracture evoked during the development of Na-free contracture. In *B* caffeine was applied for 20 s.

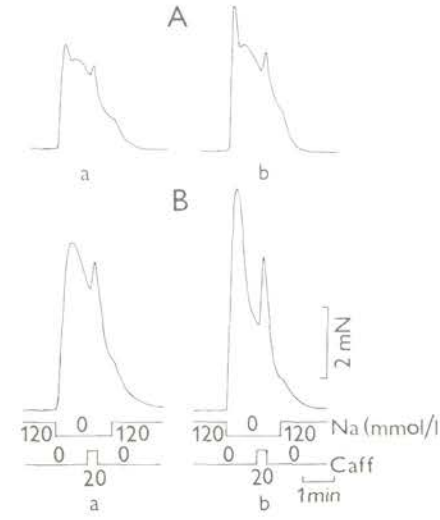


**Fig. 4.** Effects of caffeine on Na-loaded ureter muscle applied during the development of Na-free contracture with Tris (*a*) and $K^+$ (*b*) used as $Na^+$ substitute at 37 °C (*A*) and 27 °C (*B*). Note the potentiation of both the Na-free and caffeine contracture by reduced temperature. The amplitude of caffeine contractures was always higher when $K^+$ was used as Na-substitute.

*The effect of temperature on caffeine contracture*

A synergism between the effects of cooling and caffeine is well established in a number of muscles (Sakai 1965; Ito and Kuriyama 1971; Chapman and Leoty

1976). In our experiments we also found a strong potentiation of caffeine contracture by low temperature (Fig. 4*B*, *a*, *b*). Fig. 4 also shows that low temperature potentiated the low-Na contracture when both K and Tris were used as Na-substitute. Keeping the ureter muscle in Krebs solution at low temperatures (18—20 °C) led to the appearance of both low-Na and caffeine contracture. Fig. 5 shows that ureter muscle kept for 60—90 min at 21 °C responds to application of low concentration (1 mmol/l) of caffeine with transient contractures, and the maximal response was seen with 20 mmol/l of caffeine (Fig. 5*F*). The strength of the caffeine contractures of ureter muscle kept in Krebs solution at 21 °C as well as the rate of tension development and spontaneous relaxation were increased by raising the concentration of caffeine. Again, the tissue generated low-Na contractures totally insensitive to organic Ca channel blockers suggesting that in cold bath the ureter muscle accumulates Na ions and that Ca enters the cell via Na-Ca exchange. In our previous study (Aickin et al. 1984) we found that Na-loaded tissue placed in Na-free solution spontaneously relaxed and this relaxation was associated with a loss of intracellular Na ions. It was therefore of interest to see the dependence of caffeine contractures on the duration of exposure of the tissue to Na-free solution. Fig. 6 shows a decrease in the amplitude of caffeine contractures of the Na-loaded ureter muscle during its continuous perfusion with Na-free solution irrespective of the Na substitute used. In these experiments nifedipine ($10^{-5}$ mol/l) was used to exclude possible involvement of voltage operated Ca channels when K ions were used as Na substitute. Application of caffeine during the development of Na-free contracture showed a biphasic effect, i.e. the stimulatory action of caffeine was followed by the relaxant action on Na-free contracture which recovered gradually after the removal of caffeine from the bathing fluid.


**Fig. 5.** Traces of contractures evoked in the ureter muscle in normal Krebs solution at 21 °C by 1 (*B*), 2 (*C*), 5 (*D*), 10 (*E*) and 20 mmol/l (*F*) caffeine application for 20 s. Trace A represents potassium (126 mmol/l) contracture generated by the ureter at 21 °C to show the potency of the stimulatory action of caffeine.

After complete relaxation of Na-free contracture the muscle failed to generate any caffeine contracture. These results suggest that filling of the caffeine-sensitive Ca store is dependent on the entry of Ca ions via Na-Ca exchange. It was of interest to know whether this caffeine-sensitive Ca store could sequester Ca ions entering the cell via voltage operated Ca channels. To study this, the effect of caffeine on ureter muscle in isotonic K solution was investigated.

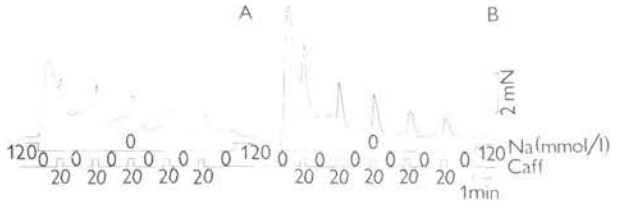


**Fig. 6.** Dependence of the strength of caffeine contractures evoked in the ureter muscle on time of development of Na-free contracture caused by continuous exposure of Na-loaded ureter to Na-free solution with Tris (*A*) and $K^+$ (*B*) used as Na substitute at 27 °C in the presence of nifedipine ($10^{-5}$ mol/l).

*Caffeine contractures evoked in isotonic K-solution*

When the ureter muscle was placed in isotonic K-solution it responded with a contracture consisting of an initial fast and a subsequent slow component (Fig. 7).

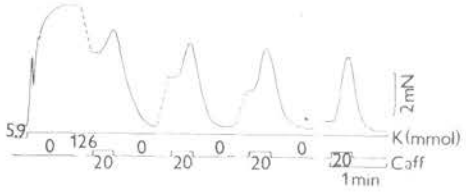


**Fig. 7.** Dependence of the strength of caffeine contractures evoked in the ureter muscle in 126 mmol-/l-K (Na-free) solution at 27 °C on time of development of tonic tension of the potassium contracture. Gaps represent 10 min intervals between the tests.

The tonic component showed time and temperature dependent decay as noted earlier (for a review, see Bolton 1979). This tonic tension was highly sensitive to Ca-channel blockers suggesting that the tonic tension is totally dependent on Ca entry via noninactivating voltage operated Ca channels (Kochemasova and Shuba 1979; Aickin et al. 1984). Application of caffeine during tonic tension evoked a transient contracture (Fig. 7). Again, the effect of caffeine on the ureter muscle in high-K solution was biphasic, i. e. the transient caffeine contracture was followed by relaxation of tonic tension which slowly recovered after the removal of caffeine from the bathing fluid. Ca-channel blockers (nifedipine, D600, diltiazem) quickly relaxed tonic tension and prevented repeated caffeine contractures. These results indicate that the entry of Ca ions into the cell is necessary to fill the caffeine-sensitive Ca store. We noted that after complete relaxation caused by Ca-antagonists the ureter muscle in isotonic K solution could respond with one submaximal contracture to caffeine application within the first 5—7 min. This implies that the caffeine-sensitive Ca store loses its Ca content if there is no Ca supply to the cell.

*Effect of $[Ca]_o$ on caffeine contracture*

The caffeine contractures of skeletal and mammalian heart muscle, in which s.r. is well developed (Frank 1962; Chapman and Leoty 1976), show little dependence on the presence of Ca ions in the bathing fluid. Removal of Ca ions from the bathing fluid with 1 mmol/l EGTA prevented the development of repeated caffeine contractures of the ureter muscle at 21 °C, althouth one contracture could still be evoked after 5 min perfusion of the ureter muscle with Ca-free solution.

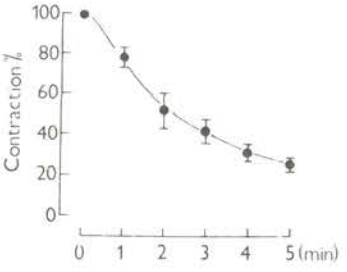


**Fig. 8.** Time course of the loss of stored $Ca^{2+}$ in Ca-free EGTA solution (1 mmol/l) at 21 °C. The size of the store is measured as the contractile response to 2 mmol/l caffeine expressed as a percentage of the control response of the tissue to 20 s application of caffeine. The bars represent S. E. of means ($n=6$).

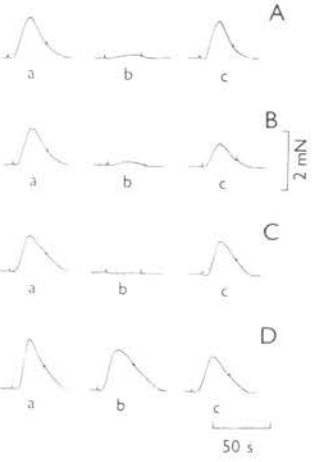


**Fig. 9.** Effects of local anaesthetics on caffeine contractures of the ureter muscle at 21 °C. Contractures were evoked by caffeine at 2 mmol/l. *A, a*; *B, a*; *C, a*; *D, a*, caffeine contractures generated by ureter muscle in the absence of local anaesthetics; *A, b*; *B, b*; *C, b*; *D, d*, the effect of benzocaine (1 mmol/l); procaine (1 mmol/l); tetracaine (0,5 mmol/l); lidocaine (1 mmol/l), respectively. The tissue was pretreated with local anaesthetics for 5 min prior to the application of caffeine. *A, c*; *B, c*; *C, c*; *D, c*, the recovery of caffeine contractures after removal of local anaesthetics from the bathing fluid.

These results suggest that Ca ions released by caffeine are not taken up by the caffeine-sensitive store. Moreover, the caffeine-sensitive Ca store normally loses its Ca content during the perfusion of the muscle with Ca-free solution as can be seen from the dependence of the amplitude of caffeine contractures on the time of tissue perfusion with Ca-free solution (Fig. 8).

*Effect of local anaesthetics on caffeine contractures*

The antagonism of the effects of caffeine by local anaesthetics in well documented (Feinstein 1963; Chapman and Leoty 1976; Itoh et al. 1981). However, this is not a general property of all local anaesthetics, since lidocaine and cocaine did not antagonize caffeine contractures (Kuriyama 1981). In our experiments we tested the effects of procaine, tetracaine, lidocaine, benzocaine and QX-314. The first three local anaesthetics were effective blockers of caffeine contractures at 21 °C, while the two latter proved to be ineffective (Fig. 9).

## Discussion

The results obtained show that caffeine has an inhibitory action on the evoked action potential and phasic contraction as well as on high-K and low-Na contractures of the ureter muscle. The inhibition of this sort noted earlier (for a review, see Bolton 1979) indicates that caffeine acts as an unspecific Ca antagonist. It was supposed that caffeine at the level of the plasma membrane interferes with Ca channels and transduction events necessary for intracellular Ca release (Leijten and van Breemen 1984).

Under untreated conditions caffeine failed to induce any contracture of the resting ureter muscle. As shown by the present experiments, ureter smooth muscle responds in Krebs solution with transient contractures to application of caffeine only when intracellular Na concentration is raised; the latter event was shown to promote Ca influx into the cell via Na—Ca exchange (Aickin et al. 1984). The strength of the caffeine contracture was always higher when caffeine was applied during the development of low-Na contracture. Caffeine contractures decreased during continuous perfusion of the tissue with Na-free solution, resulting in spontaneous relaxation of the low-Na contracture associated with a loss of intracellular Na ions (Aickin et al. 1984), and eventually totally disappeared after complete relaxation of the low-Na contracture. This strongly suggests that Ca ions entering the cell via Na—Ca exchange are accumulated in the caffeine-sensitive store in quantities sufficient to evoke submaximal contracture even in the virtual absence of $[Ca]_o$. This means that the caffeine-sensitive store of the ureter muscle sequesters, at least temporarily, quite large amounts of Ca ions entering the cell via Na—Ca exchange. Ca blockers effectively blocked phasic and tonic contractions of the ureter muscle associated with the influx of Ca into the cell via voltage operated Ca channels, but had no effect on the low-Na contracture (Aickin et al. 1984). This explains the ability of the ureter muscle to generate repeated caffeine contractures in the presence of Ca blockers as long as the intracellular concentration of Na ions promoting Ca influx remains high.

Also, caffeine-sensitive Ca store can accumulate Ca ions entering the cell via voltage operated Ca channels since the ureter muscle placed in isotonic K-solution is

able to respond with transient contractures to caffeine application. Blockade by Ca antagonists of the Ca influx via noninactivating Ca channels opened during sustained depolarization prevented the occurrence of repeated caffeine contractures; this again implies a strong dependence of filling of the caffeine-sensitive Ca store on the influx of Ca ions into the cell. Nevertheless, the ability of caffeine to evoke a full-sized single transient contracture of the ureter muscle placed in Ca-free solution suggests that, unlike phasic, potassium and low-Na contractions which are all totally dependent on the presence of $[Ca]_o$ (Aickin et al. 1984), caffeine contractures are not directly dependent on the Ca influx but require increased $[Ca]_i$ that is provided by either the Na—Ca exchange mechanism or voltage operated Ca channels. In this respect the ureter muscle closely resembles frog heart in which caffeine also initiated contractures when the muscle has already developed tension in either high-K or Na-free solution (Chapman and Miller 1974).

These results strongly suggest that Ca ions which irrespective of the mechanism enter the cell are sequestered by the caffeine-sensitive Ca store. Since caffeine is known to specifically interfere with Ca movement in s.r. (Weber 1968; Thorpe and Seeman 1971), it might be concluded that the ureter muscle possesses s.r. which serves as a temporal Ca sink but not as the Ca storage site. The relatively quick abolishment of caffeine contractures of the ureter muscle placed in Ca-free solution along with the inability of caffeine to evoke repeated contractures during continuous perfusion with Ca-free solution also supports the idea that Ca influx is a prerequisite for Ca-loading of ureter muscle s.r. The transient nature of caffeine contractures, even in the presence of $[Ca]_o$, means that Ca ions released by caffeine are removed from the cytoplasm by a mechanism other than Na—Ca exchange, since a reversal of the exchanger is actually needed to fill s.r. The existence of spontaneous relaxation of caffeine contracture led to the idea of non-homogeneity of the relaxing system in other muscles (Winegrad 1970; Caputo 1972; Chapman and Miller 1971; Chapman and Leoty 1976). Chapman and Miller (1971) suggested the presence of two relaxing systems in the frog heart or two stages in the removal of Ca ions from the sarcoplasm, with the cell membrane as a second stage providing a back-up system for s.r. Evidence for the existence of a metabolically dependent Ca-pump in smooth muscle sarcolemma was presented by a number of authors (for a review, see Kuriyama 1981). Cooling which significantly potentiates caffeine contracture is known to affect the relaxation rate of various smooth muscles (Scripnyuk and Burdyga 1977; Droogmans and Casteels 1981). The high value of $Q_{10}$ ($\geq 3$) for relaxation suggests that the removal of Ca ions from the sarcoplasm is an active process (Chapman and Leoty 1976; Scripnyuk and Burdyga 1977; Droogmans and Casteels 1981). A good parallelism between the slowing of the relaxation and extrusion of $^{45}Ca$ in the rabbit aortae caused by cooling suggested the existence of metabolically dependent Ca-pump in the

sarcolemma (Droogmans and Casteels 1981). The strong dependence of Ca gradient on the cellular ATP content found in visceral and vascular smooth muscle is also in favour of the existence of membrane-bound Ca pump in smooth muscles (van Breemen et al. 1973). Since low temperature potentiated caffeine contracture and contractures evoked by low-Na and high-K solution, it may be assumed that Ca ions either released by caffeine or entering the cells via Na—Ca exchange or voltage operated Ca channels, are extruded from the cell by membrane-bound Ca-pump less effectively and more fully activate the contractile machinery of the muscle. However, as suggested by the present experiments, the role of s.r. in the regulation of the cytoplasmic concentration of Ca ions in the ureter muscle is also important. Yet, it is not clear whether the s.r. Ca-pump and Ca-pump of the plasma membrane work in parallel or in series, and this requires further elucidation.

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