*J. Physiol.* (1975), **246**, *pp.* 197–211
*With* 6 *text-figures*
*Printed in Great Britain*



# ACTIVE CHLORIDE SECRETION BY *IN VITRO* GUINEA-PIG SEMINAL VESICLE AND ITS POSSIBLE RELATION TO VESICULAR FUNCTION *IN VIVO*

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(*Received 19 July 1974*)

SUMMARY

1. The guinea-pig seminal vesicle *in vivo* is characterized by a transmural electrical potential difference of approximately 11 mV with the lumen electrically negative with respect to the interstitial fluid. The concentrations of Na, Cl and K in the vesicular fluid are 13, 15 and 0·4 mM, respectively.

2. When mounted as a flat sheet in a short-circuit apparatus, guinea-pig seminal vesicles initially undergo a decline in the transmural electrical potential difference and short-circuit current ('low phase') followed by a spontaneous increase in these parameters ('high phase').

3. During the low phase, net Cl movements across the tissue do not differ significantly from zero, and there is a small 'residual' current that is unaccounted for.

4. During the high phase, there is a significant active Cl secretion into the mucosal solution, no detectable net movement of Na and an unaccounted for or 'residual' current that is equal to that found in the low phase.

5. Theophylline, dibutyryl-3′-5′ cyclic adenosinemonophosphate, prostaglandin $E_1$ and prostaglandin $F_{2\alpha}$ markedly stimulate the transmural electrical potential difference and short-circuit current during the low phase, but have no effect when added to the bathing solution during the high phase.

6. Diffusion potentials determined across *in vitro* seminal vesicles suggest that the spontaneous transmural electrical potential difference *in vivo* may be attributable to the large ionic asymmetries between the vesicular fluid and the plasma.

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7. It is postulated that two phases are involved in the elaboration of seminal vesicular fluid. The initial phase, following emptying of the vesicle, is characterized by the secretion of electrolytes, organic molecules and water. Active Cl secretion presumably regulated by intracellular cyclic adenosinemonophosphate and/or prostaglandins may be the driving force for this initial secretion of electrolytes. Following this secretory phase, electrolytes and water are reabsorbed, thereby concentrating the organic components in the vesicular reservoir.

## INTRODUCTION

The concentrations of Na, K and Cl in guinea-pig seminal vesicular fluid are much lower than those in either the vesicular tissue or the plasma (Breuer & Whittam, 1957). However, although it has been shown that the epithelial cells of guinea-pig seminal vesicle can maintain steady-state intracellular ion concentrations *in vitro* under both aerobic and anaerobic conditions providing suitable substrates are present in the incubation medium (Breuer & Whittam, 1957; Whittam & Breuer, 1959), the trans-epithelial transport processes responsible for the elaboration of the vesicular fluid have not been investigated.

The purpose of the present study was to examine Na and Cl transport across an *in vitro* preparation of guinea-pig seminal vesicle employing the short-circuit technique originally described by Ussing & Zerahn (1951). In addition, the possible roles of intracellular 3′-5′ cyclic adenosinemonophosphate (cyclic AMP) and prostaglandins in regulating these transport processes were explored. The guinea-pig is especially useful for these investigations because its seminal vesicle is a tubular structure, approximately 5–8 cm long, that is quite amenable to experimental manipulation both *in vivo* and *in vitro*.

## METHODS

Guinea-pigs weighing between 600 and 900 g were anaesthetized with I.P. injection of pentobarbitone.

*In vivo measurements.* The seminal vesicle was exposed through a small suprapubic incision. The transmural electrical potential difference was determined by inserting a sharpened glass electrode containing either 3 M-KCl or the standard Ringer solution into the lumen of the vesicle. A second glass electrode containing the same solution was inserted subcutaneously. The electrodes were connected via salt bridges to matched calomel electrodes which, in turn, were connected to a Keithley 600 A high impedance electrometer.

Vesicular fluid was obtained from anaesthetized animals by inserting a sharpened glass cannula into the vesicle and gently aspirating some of the luminal contents. Na and K concentrations were determined on suitably diluted aliquots of vesicular fluid using an Instrumentation Laboratories flame photometer with internal lithium standard; Cl was determined using a Buchler-Cotlove chloridometer.

*In vitro measurements.* The basic techniques and apparatus for measuring the

transmural electrical potential difference ($\psi_{ms}$), the short-circuit current ($I_{sc}$) and transmural ion fluxes have been described in detail previously (Schultz & Zalusky, 1964). Briefly, guinea-pigs were anaesthetized; both seminal vesicles were slit open to form a flat sheet; the contents were washed out, and a section of tissue was mounted between two plexiglass half-chambers. The area of tissue exposed to the mucosal and serosal bathing solutions was 0·8 $cm^2$ and was rectangular in shape, having a width of 0·4 cm and a length of 2 cm. The $\psi_{ms}$ and $I_{sc}$ were determined using agar-electrodes that were inserted into both half-chambers. In most experiments, both surfaces of the tissues were bathed by a Ringer–phosphate buffer (pH 7·4) (Umbreit, Burris & Stauffer, 1949) and gassed with 100 % $O_2$. In some experiments, Cl and Na fluxes were determined using a Ringer–bicarbonate buffer (25 mM-$HCO_3$; pH 7·4) gassed with 5 % $CO_2$–95 % $O_2$. In all experiments, the solutions contained 10 mM glucose and either 10 mM Na glutamate or 10 mM glutamine. The bathing solutions were always maintained at 37° C.

Specific fluid resistance averaged 40 Ω/cm and all measurements of $I_{sc}$ were corrected for this resistance, which did not exceed 15 % of the total resistance of fluid *plus* tissue. The electrical potential difference was corrected for small electrode asymmetries which never exceeded 0·3 mV.

Bidirectional transmural fluxes were determined on short-circuited, paired vesicles from the same animal using $^{36}Cl$ and $^{22}Na$. Either $^{36}Cl$ or $^{22}Na$ was added to the solution bathing the serosal side of one vesicle and the mucosal side of the other, and the rate of appearance of isotope in the initially unlabelled side was determined using a liquid scintillation spectrometer. Following the addition of isotope at least 20 min were allowed to elapse before the onset of sampling in order to ensure the achievement of a steady state; thereafter, three to four 0·1 ml. samples were collected at 10-min intervals during phases when the $\psi_{ms}$ and $I_{sc}$ were relatively constant. Net transmural fluxes were calculated from the differences between the two oppositely directed unidirectional fluxes.

Diffusion potentials were measured in three vesicles in order to determine the extent to which ionic asymmetries between the vesicular fluid and the plasma may contribute to the transmural electrical potential difference *in vivo*. Tissues mounted between plexiglass half-chambers were bathed by the standard Ringer–phosphate buffer containing $10^{-3}$ M ouabain, so that within 30 min the spontaneous $\psi_{ms}$ and $I_{sc}$ did not differ significantly from zero. The mucosal solution was then progressively diluted with an isotonic 300 mM solution of mannitol and the resultant $\psi_{ms}$ was determined following each dilution after ascertaining that the value was constant for at least 3 min. KCl-agar electrodes (3 M) were employed to measure the $\psi_{ms}$ in these experiments in order to minimize the magnitude of asymmetric junction potentials. Because the volumes of the bathing solutions were relatively large (10 ml.), diffusion of KCl from the bridges did not significantly alter the composition of the bathing media.

In a few experiments either theophylline, dibutyryl-3′-5′ cyclic AMP, prostaglandin $E_1$ or prostaglandin $F_{2\alpha}$ were added to the bathing solutions at various times after the tissue was mounted between the half-chambers.

$^{36}Cl$ and $^{22}Na$ were obtained from the New England Nuclear Corp.; theophylline was obtained from K & K Laboratories; dibutyryl-cyclic AMP was obtained from Sigma Chemicals; and prostaglandins were a generous gift from Dr John Pike of the Upjohn Company. All other reagents were of the highest purity commercially available.

All results are expressed as the mean ± S.E. of the mean based on the number of tissues examined ($n$).

## RESULTS

*Transmural electrical potential difference and electrolyte concentrations in vivo.* The lumen of the seminal vesicle is 11·1 ± 2·3 mV ($n$ = 6) electrically negative with respect to the interstitial fluid. The Na, Cl and K concentrations of the vesicular fluid, determined on twelve animals, averaged 13·1 ± 1·1, 14·5 ± 0·5 and 0·4 ± 0·1 mM, respectively. Thus, the sum of the Na and K concentrations does not differ from that of Cl. Similar values for guinea-pig vesicular fluid were reported by Breuer & Whittam (1957).

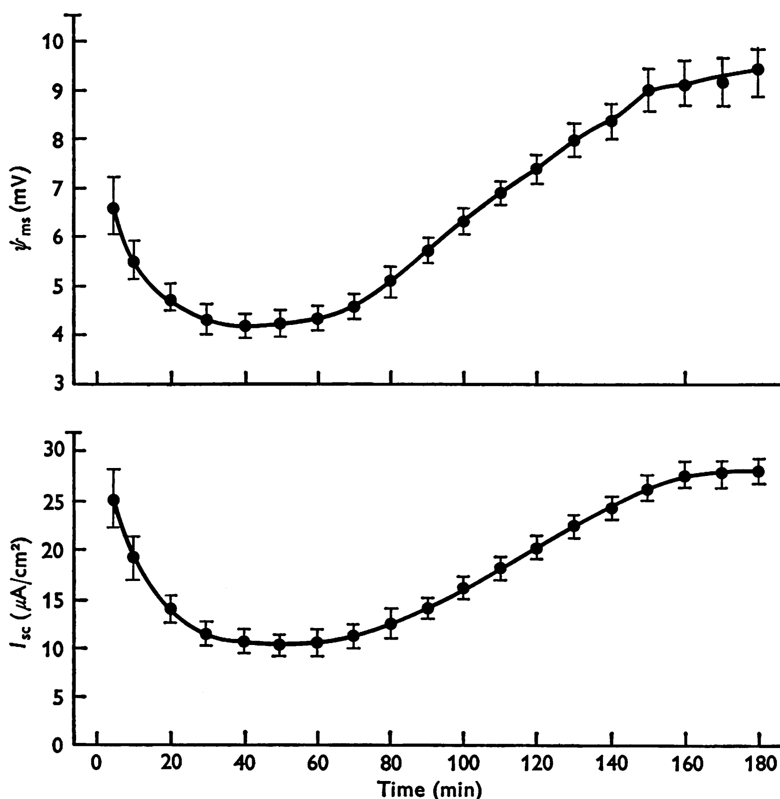


Fig. 1. Time-course of the transmural electrical potential difference ($\psi_{ms}$) and short-circuit current ($I_{sc}$) across isolated seminal vesicles in phosphate buffer. Values shown are the means (± S.E.) obtained from twenty-six tissues.

In view of the fact that the plasma concentration of Na, Cl and K in the guinea-pig are approximately 130, 100 and 6 mM, respectively, the steady-state concentrations of these ions in the vesicular fluid do not conform with a passive (or equilibrium) distribution calculated from the

Nernst equation. Thus, the concentration differences between lumen and plasma must be the result of one or more active ion-transport processes.

*Electrical parameters and ion fluxes in vitro.* The transmural electrical potential difference ($\psi_{ms}$) and $I_{sc}$ across seminal vesicle mounted as a flat sheet between two half-chambers and bathed on each surface with an identical Ringer–phosphate buffer are illustrated as a function of time in Fig. 1. Both the $\psi_{ms}$ and $I_{sc}$ decrease for the first 60 min after mounting; thereafter, both increase and reach fairly stable levels by about 140 min. These stable values are generally maintained for approximately 1 to 2 hr and will be referred to as the 'high phase'. The tissue resistance during this high phase is approximately 300 Ω cm².

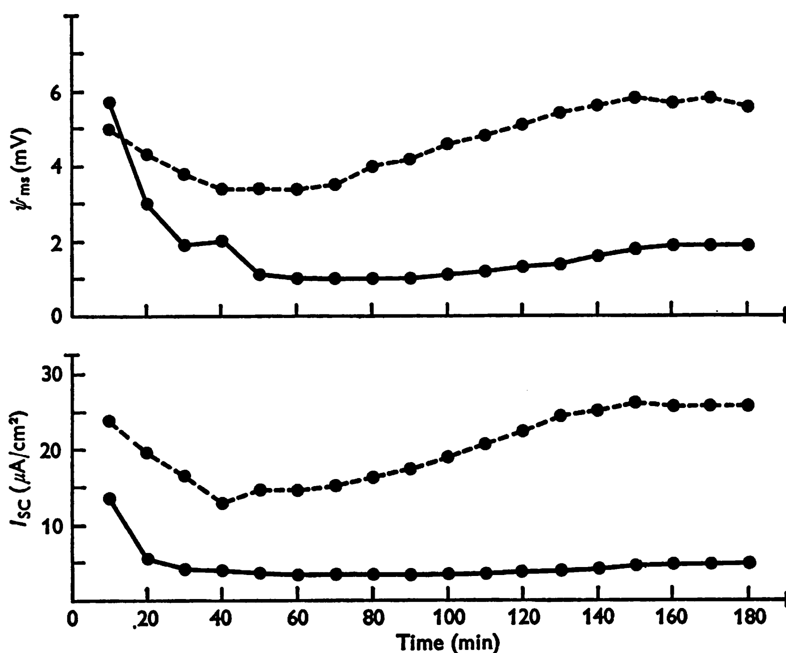


Fig. 2. Transmural electrical potential difference ($\psi_{ms}$) and short-circuit current ($I_{sc}$) in chloride-free buffer (solid lines) and standard phosphate buffer (dashed lines). Typical result of five experiments on paired vesicles from the same animals.

When Cl in the buffer is replaced isotonically with $SO_4$ plus mannitol, both the $\psi_{ms}$ and $I_{sc}$ remain at very low levels, exhibiting only a comparatively small rise during the same period when the $\psi_{ms}$ and $I_{sc}$ of the companion tissue from the same animal undergo their normal increases (Fig. 2). Similarly, when Na is replaced with choline, both the $\psi_{ms}$ and $I_{sc}$ remain close to zero (Fig. 3).

The transmural fluxes of Cl under short-circuit conditions determined between 30 and 50 min ('low phase') and after 140 min (high phase) are given in Table 1. During the low phase, there is a small but statistically insignificant net flux of Cl from the serosal to the mucosal solutions (Cl secretion). The average $\psi_{ms}$ and $I_{sc}$ for the two groups of tissues employed for the measurement of the oppositely directed Cl fluxes did not differ significantly and the mean $I_{sc}$ for all of the tissues was $0{\cdot}4 \pm 0{\cdot}1$ $\mu$equiv/hr.cm$^2$. Clearly, this cannot be attributed to net Cl transport since it is essentially zero.

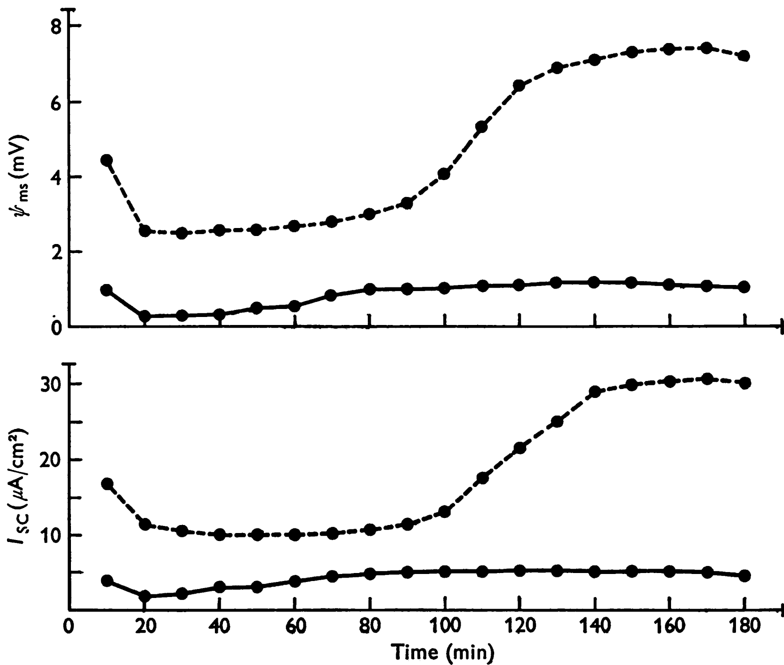


Fig. 3. Transmural electrical potential difference ($\psi_{ms}$) and short-circuit current ($I_{sc}$) in sodium-free buffer (continuous line) and standard phosphate buffer. Typical result of five experiments on paired vesicles from the same animals.

During the high phase the net flux of Cl into the lumen increased significantly to $0{\cdot}8 \pm 0{\cdot}2$ $\mu$equiv/hr.cm$^2$. The mean $I_{sc}$ for the two groups of tissues during the high phase is $1{\cdot}2 \pm 0{\cdot}1$ $\mu$equiv/hr.cm$^2$. Thus, the increase in net Cl secretion accounts for 85 % of the increase in $I_{sc}$ (i.e. 0·6 $\mu$equiv/hr.cm$^2$ compared to 0·7 $\mu$equiv/hr.cm$^2$). As in the low phase, the high phase is also characterized by a residual current of approximately 0·4 $\mu$equiv/hr.cm$^2$ that is not attributable to active Cl secretion.

TABLE 1. Transmural Cl fluxes

| | $n$ | $J_{sm}$ ($\mu$equiv/hr.cm²) | $I_{sc}$ ($\mu$equiv/hr.cm²) | $\psi_{ms}$ (mV) | $J_{ms}$ ($\mu$equiv/hr.cm²) | $I_{sc}$ ($\mu$equiv/hr.cm²) | $\psi_{ms}$ (mV) | $J_{net}$ ($\mu$equiv/hr.cm²) |
|---|---|---|---|---|---|---|---|---|
| Low phase; phosphate buffer | 6 | $3.0 \pm 0.2$ | $0.4 \pm 0.1$ | $5.2 \pm 0.7$ | $2.9 \pm 0.2$ | $0.4 \pm 0.1$ | $5.4 \pm 0.3$ | $0.2 \pm 0.3$ |
| High phase; phosphate buffer | 9 | $3.9 \pm 0.1$* | $1.2 \pm 0.1$* | $9.0 \pm 0.3$* | $3.1 \pm 0.1$ | $1.1 \pm 0.1$* | $8.8 \pm 0.3$* | $0.8 \pm 0.2$† |
| High phase; bicarbonate buffer | 6 | $3.6 \pm 0.1$* | $0.9 \pm 0.1$* | $6.8 \pm 0.3$* | $3.2 \pm 0.1$ | $0.9 \pm 0.1$* | $7.0 \pm 0.5$* | $0.4 \pm 0.1$† |

$J_{sm}$ is the flux from serosal solution to the mucosal solution; $J_{ms}$ is the flux from the mucosal solution to the serosal solution; $J_{net} = J_{sm} - J_{ms}$. $n$ = the number of experiments on paired tissues. The average values for $\psi_{ms}$ and $I_{sc}$ for each of the paired tissues are given.

* Significantly different from low phase at $P < 0.01$ using Student's $t$ test.

† Significantly different from zero at $P < 0.01$ using paired $t$ test.

TABLE 2. Transmural Na fluxes

| | $n$ | $J_{sm}$ ($\mu$equiv/hr.cm²) | $I_{sc}$ ($\mu$equiv/hr.cm²) | $\psi_{ms}$ (mV) | $J_{ms}$ ($\mu$equiv/hr.cm²) | $I_{sc}$ ($\mu$equiv/hr.cm²) | $\psi_{ms}$ (mV) | $J_{net}$ ($\mu$equiv/hr.cm²) |
|---|---|---|---|---|---|---|---|---|
| High phase; phosphate buffer | 5 | $1.3 \pm 0.1$ | $1.1 \pm 0.1$ | $9.3 \pm 0.6$ | $1.5 \pm 0.1$ | $1.1 \pm 0.1$ | $10.4 \pm 0.5$ | $-0.2 \pm 0.1$ |
| High phase; bicarbonate buffer | 4 | $2.2 \pm 0.1$ | $0.8 \pm 0.1$ | $6.3 \pm 0.4$ | $2.1 \pm 0.1$ | $0.8 \pm 0.1$ | $7.1 \pm 0.6$ | $0.1 \pm 0.2$ |

See legend to Table 1.

Also given in Table 1 are the Cl fluxes determined during the high phase in the presence of a Ringer–bicarbonate buffer. There is a significant net flux into the mucosal solution of $0 \cdot 4 \pm 0 \cdot 1$ $\mu$equiv/hr. cm$^2$. This is 0·4 $\mu$equiv/hr. cm$^2$ lower than that observed using the Ringer–phosphate buffer. Similarly, the mean $I_{sc}$ in the presence of the Ringer–bicarbonate buffer ($0 \cdot 9 \pm 0 \cdot 1$ $\mu$equiv/hr. cm$^2$) is 0·3 $\mu$equiv/hr. cm$^2$ lower than that observed in the presence of the Ringer–phosphate buffer. Thus, in spite of the fact that the $I_{sc}$ underwent a greater spontaneous increase in the presence of phosphate than in the presence of bicarbonate, it is clear from these data that the increase in the presence of both buffers is for the most part attributable to active Cl secretion from the serosal solution into the mucosal solution. Finally, in the bicarbonate buffer as in the phosphate buffer there is still approximately 0·4 $\mu$equiv/hr. cm$^2$ of current unaccounted for by net Cl secretion.

In view of the fact that the tissue must be characterized by the net movement of a charged species other than Cl during the low phase and high phase, bidirectional Na fluxes were determined. As shown in Table 2, the net Na flux under short-circuit conditions does not differ significantly from zero during the high phase in the presence of either a phosphate buffer or a bicarbonate buffer, and thus cannot contribute to the short-circuit current.

*Diffusion potentials.* As noted above, the concentrations of Na, Cl and K in vesicular fluid obtained from anaesthetized guinea-pigs are approximately one tenth those in the plasma, and the lumen of the vesicle is approximately 10 mV electrically negative with respect to the interstitial fluid. It was, therefore, of interest to examine the passive properties of this tissue in order to determine the extent to which diffusion potentials resulting from the ionic asymmetries may contribute to the transmural electrical potential difference *in vivo*. However, because the tissue is characterized by a spontaneous $\psi_{ms}$ *in vitro* even when both bathing solutions have identical compositions, and because this electrical potential difference is influenced by the Cl and Na concentrations in the bathing media (Figs. 2 and 3), it was first necessary to abolish the spontaneous $\psi_{ms}$. As shown in Fig. 4, the addition of $10^{-3}$ M ouabain to the bathing solutions abolished the spontaneous $\psi_{ms}$ when both bathing solutions had identical compositions (intercept on the abscissa); this effect was noted within 30 min. Subsequent progressive dilution of the mucosal solution alone with isotonic mannitol solution resulted in diffusion potentials oriented so that with decreasing NaCl concentration in the mucosal solution, this solution became increasingly electrically negative with respect to the serosal solution. Assuming that ouabain does not alter the permselective properties of this tissue, these results indicate that the permea-

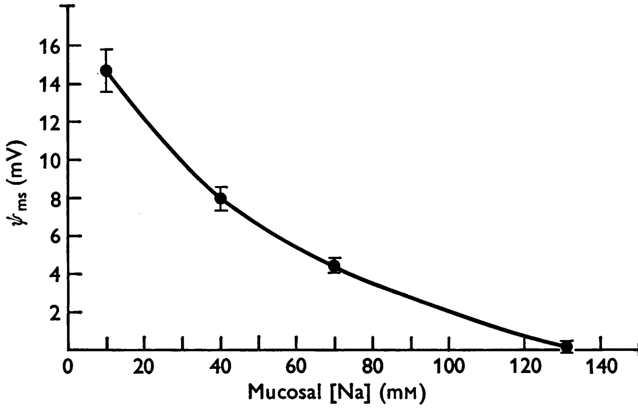


Fig. 4. Diffusion potentials across seminal vesicles poisoned with $10^{-3}$ M ouabain as a function of the Na concentration in the mucosal solution.

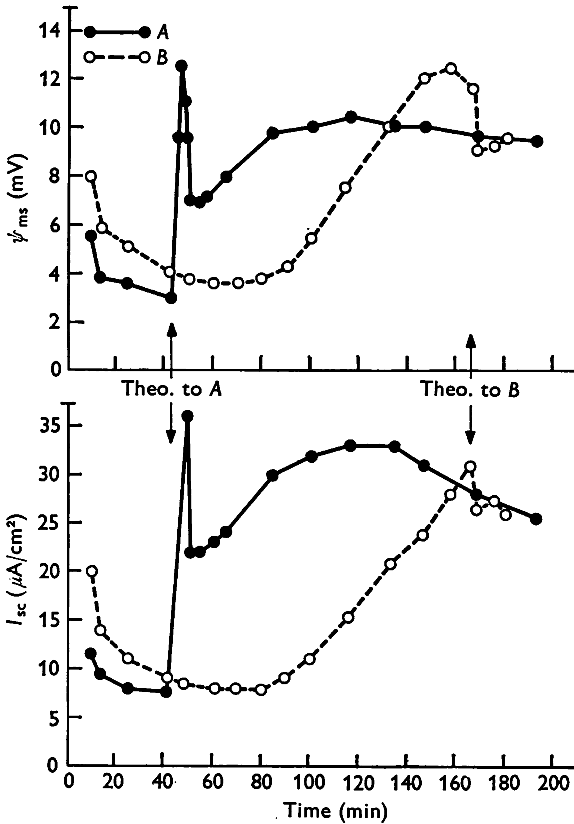


Fig. 5. Effects of 10 mM theophylline on the $\psi_{ms}$ and $I_{sc}$ following addition to the mucosal solution during the low phase ($A$) or the high phase ($B$). Typical result of five experiments on paired vesicles from the same animals.

bility of the tissue to Cl is greater than the permeability to Na, a finding that is consistent with the observation that the bidirectional fluxes of Cl exceed those of Na (Tables 1 and 2). Further, when the concentration of NaCl in the mucosal solution approximates that found *in vivo* (*ca.* 15 mM), the diffusion potential observed *in vitro* (*ca.* 13 mV) does not differ markedly from transmural electrical potential difference recorded *in vivo*. The possible implications of these findings will be discussed below.

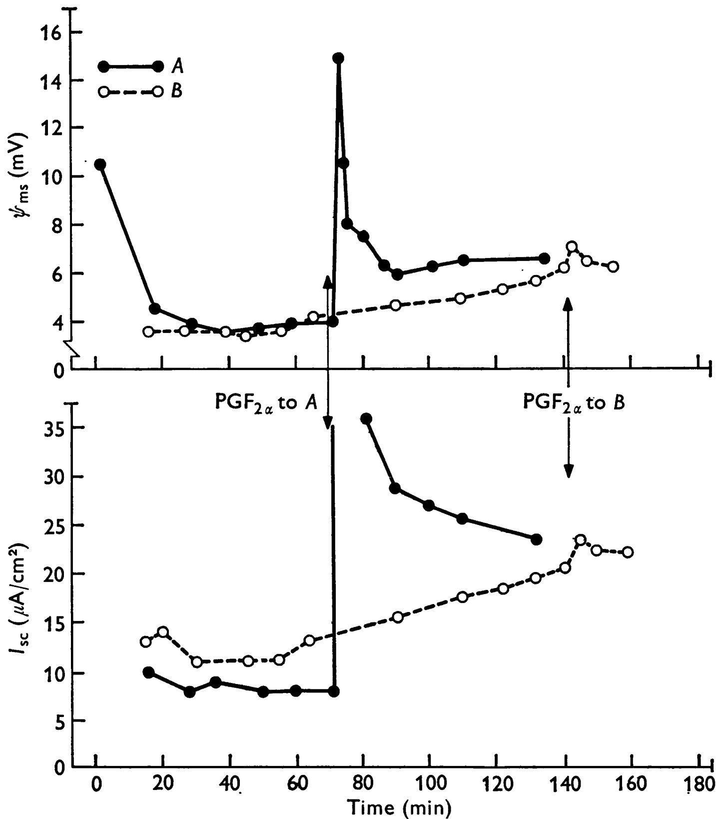


Fig. 6. Effects of prostaglandin $F_{2\alpha}$ 200 ng/ml. on $\psi_{ms}$ and $I_{sc}$ following addition to the mucosal solution during the low phase (*A*) or the high phase (*B*). Typical result of ten experiments on paired vesicles from the same animals.

*Effects of theophylline, dibutyryl-cyclic AMP and prostaglandins.* The effects of addition of 10 mM theophylline to the mucosal bathing solution alone on $\psi_{ms}$ and $I_{sc}$ are shown in Fig. 5. Clearly, when this phosphodiesterase inhibitor is added during the low phase, there is a prompt increase in $\psi_{ms}$ which is closely paralleled by an increase in $I_{sc}$. However,

when theophylline is added to the mucosal solution during the high phase, no increases in $\psi_{ms}$ or $I_{sc}$ are observed. Further, at 180 min the $\psi_{ms}$ and $I_{sc}$ of tissues treated with theophylline during the low phase do not differ from those of tissues exposed to theophylline during the high phase. Qualitatively similar results are observed following addition of 7·5 mM dibutyryl-cyclic AMP to the serosal solution.

As shown in Fig. 6, similar effects on $\psi_{ms}$ and $I_{sc}$ are observed following the addition of prostaglandin $F_{2\alpha}$ (200 ng/ml.) to the mucosal solution. Pronounced increases in both $\psi_{ms}$ and $I_{sc}$ are observed when the prostaglandin is added during the low phase, but virtually no effect is seen when it is added during the high phase. Similar results were obtained using prostaglandin $E_1$.

Finally, it should be noted that following the addition of theophylline, dibutyryl-cyclic AMP, or the prostaglandins during the low phase, the $\psi_{ms}$ and $I_{sc}$ display a pronounced 'overshoot' and then decline to values approaching those observed during the spontaneously attained high phase.

## DISCUSSION

This paper is concerned with the investigation of Na and Cl transport by *in vitro* guinea-pig seminal vesicle in an attempt to gain insight into the mechanisms responsible for producing vesicular fluid *in vivo*. The short-circuit technique which has been applied successfully to the study of ion transport across a wide variety of epithelia is ideally suited to this end. When an *in vitro* preparation of tissue is bathed on both surfaces with solutions of identical ionic composition and when the spontaneous transmural electrical potential difference is abolished by the passage of an external current across the tissue, all driving forces for transmural ionic diffusion are abolished. Under these conditions, the net movement of any ion in either direction cannot be attributed to differences in electrochemical potential so that the detection of such movement is strong, presumptive evidence that the ion in question is transported by an energy-dependent active process. Finally, the magnitude of the external current necessary to abolish the spontaneous electrical potential difference (expressed either as $\mu A/cm^2$ or $\mu$equiv/hr.$cm^2$ where 26·8 $\mu A/cm^2$ = 1 $\mu$equiv/hr) is a measure of the algebraic sum of all transmural ionic currents that cannot be attributed to simple diffusion.

The results obtained indicate that immediately after mounting the tissue, net Cl movement does not differ significantly from zero; however, approximately 60–80 min following the removal of the vesicle from the anaesthetized animal, there is a significant active secretion of Cl from the serosal solution into the mucosal solution. No net movement of Na under

short-circuit conditions is detectable; however, a small component that is statistically obscured by experimental variance cannot be excluded. These results resemble those recently reported by Brunton & Brinster (1971) for a short-circuited, *in vitro* preparation of rabbit oviduct. In addition, both the high phases and low phases are characterized by an unaccounted-for current (a 'residual current') that is consistent with the active secretion of an anion(s) other than Cl, the active absorption of a cation(s) other than Na or some combination of these movements. The specific ionic movements responsible for this residual current remain to be elucidated. Finally, although additional studies of Cl fluxes are necessary in order to establish this point with certainty, it seems likely that an elevation in intracellular cyclic AMP and/or prostaglandins stimulates active Cl secretion and accelerates the transition from the low phase (first 60–80 min after mounting) to the high phase. It is of interest in this respect that cyclic AMP and/or prostaglandins have recently been shown to stimulate active Cl secretion in a number of diverse epithelia such as rabbit ileum (Field, 1971; Al-Awqati & Greenough, 1972), gastric mucosa (Harris, Nigon & Alonso, 1969), cornea (Beitch, Beitch & Zadunaisky, 1973; Zadunaisky, Lande, Chalfie & Neufeld, 1973).

These results raise a number of questions regarding seminal vesicle function *in vivo*. The concentrations of Na, Cl and K in the vesicular fluid and the spontaneous transmural electrical potential difference indicate conclusively that one or more active reabsorptive processes must be operative *in vivo* to extrude Na, Cl and K which diffuse into the lumen from the plasma and maintain a steady-state intraluminal composition; certainly, active Cl secretion into the lumen is precluded by these data. In addition, if the diffusion potentials observed across ouabain-treated tissue *in vitro* are applicable to the *in vivo* situation, these data imply that the spontaneous electrical potential difference *in vivo* is largely a diffusion potential across an anion-selective barrier. If the *in vivo* electrical potential difference is solely due to diffusion potentials resulting from the marked ionic asymmetries, it follows that the active reabsorptive processes must be electrically neutral. In short, it would appear that the active Cl secretory process and the $\psi_{ms}$ observed during the high phase *in vitro* cannot reflect the situation that prevails *in vivo*; the close agreement between the transmural electrical potential differences under these conditions appears to be coincidental. What then is the meaning of the present *in vitro* observations? One possibility is that removal of the vesicle from *in situ* neural and humoral influences completely alters its transport function(s) and that the *in vitro* results have no physiological significance. However, it seems unlikely that simply removing the seminal vesicle would result in the *de novo* development of a Cl secretory capacity that is presumably influenced

by cyclic AMP and prostaglandins. A more attractive explanation for our findings is that active Cl secretion represents a regulated physiological transport process that is responsible for an initial phase in the elaboration of the final vesicular fluid. The guinea-pig seminal vesicle may actually secrete two different solutions during this initial phase; one a large-volume electrolyte solution and the other a relatively small-volume solution containing proteins and small organic molecules such as fructose. Support for this notion derives from the observations of Lockwood & Williams-Ashman (1971) that cholinergic drugs stimulate the secretion of alkaline phosphatase by the guinea-pig seminal vesicle while dibutyryl-cyclic AMP, theophylline and adrenergic drugs were ineffective. In our experiments, dibutyryl-cyclic AMP and theophylline increased the $I_{sc}$ and presumably elicited active Cl secretion during the low phase when the vesicle was not spontaneously secreting Cl, and in a few preliminary experiments we found that cholinergic drugs had no effect on the $\psi_{ms}$ or $I_{sc}$. Thus, it is quite possible that like the pancreas, two different secretions elicited by separate stimuli are involved in the initial elaboration of the vesicular fluid. Further, we might postulate that after the initial phase of formation of the fluid, the active Cl secretory process is inhibited and processes responsible for active Na, K and Cl reabsorption are elicited. The substantial amounts of small organic molecules, such as fructose, in the lumen would retain their osmotic equivalent of water so that the concentrations of Na, Cl and K in the lumen could fall to quite low levels. A steady state would be achieved when the rate of ionic diffusion from plasma to lumen equals the rate of active reabsorption. In short, we postulate that after the vesicle has been emptied by ejaculation, ions, organic compounds and water are secreted into the lumen. Active Cl secretion presumably regulated by cyclic AMP and/or prostaglandins may be the principal driving force for the secretion of electrolytes and their osmotic equivalent of water. Subsequently, the stimulus for active Cl secretion is inhibited (perhaps by the accumulation of some inhibitory substance in the lumen) and ion-reabsorptive processes come into play. This would reduce the overall volume of the vesicle, prevent the generation of excessive pressures, and at the same time concentrate the essential vesicular components that contribute to the spermatic fluid. Following ejaculation, the active Cl secretory process, possibly stimulated by cyclic AMP and/or prostaglandins, would again function to rapidly restore vesicular fluid. Thus, the delayed onset of active Cl secretion observed in the present study may reflect the physiological response to emptying of the seminal vesicle.

This admittedly speculative explanation has several physiological analogues in systems that display secretory and storage functions. For example, in the hepato-biliary system, water and electrolytes secreted

by the bile canaliculi are reabsorbed by the gall bladder, resulting in a highly concentrated solution of bile salts and preventing the accumulation of excess volume with an attendant increase in pressure. Further, the rete testis of the rat secretes a relatively large volume of NaCl solution that is almost entirely reabsorbed in the caput epididymis, and active Cl transport appears to be implicated in this reabsorptive process (Levine & Marsh, 1971; Tuck, Setchell, Waites & Young, 1970). One difference between these examples and our proposal is that for the case of the seminal vesicle, a single structure would serve both as the secretory organ and as the reabsorptive and storage organ. This would necessitate the presence of different cell types (secretory and absorptive cells) that are subject to neural and/or humoral regulation *or* a single cell type that is capable of secretion as well as absorption of electrolytes and whose function is regulated by intracellular factors. More sophisticated histological studies than are currently available could assist in resolving this issue.

In summary, we have demonstrated a hitherto unsuspected system in the guinea-pig seminal vesicle which is capable of active Cl secretion and appears to be regulated by intracellular cyclic AMP and/or prostaglandins. Whether the function of this system is as we have speculated above must await further investigation. For example, we are unaware of any studies on the electrolyte composition of vesicular fluid in sexually active or repeatedly ejaculated animals compared to sexually inactive ones, nor are we aware of any studies on possible changes in the composition of vesicular fluid during its storage in the vesicle. Such studies could shed light on the possible physiological significance of the present findings. Finally, whether these findings are unique to the guinea-pig seminal vesicle or mimic the behaviour of seminal vesicles of other species must also await further study.

This investigation was supported by research grants from the Population Council (M71.065C) and the U.S.P.H.S., National Institute of Arthritis, Metabolism and Digestive Diseases (AM16275-02). Dr Levine was a Postdoctoral Research Fellow of the Population Council. Mrs Rinaldo's work on this project was sponsored by the Summer Research Program of the Medical Alumni Association of the University of Pittsburgh School of Medicine.

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