this communication, the orientation of telomeric C-bands in Allium cepa, indicating non-random arrangement of chromosomes in interphase cells, will be presented.

*Material and methods.* Chromosome and interphase cell preparations were made from roottip meristems of Allium cepa. The preparations were treated with saturated solution of barium hydroxide for 5 min and were allowed to renaturate in 2X SSC at 66 °C for 2 h. The preparations were stained in 5% Giemsa (Merck) solution; washed, air dried and mounted in euparal The method is a slight modification of Vosa and Marchi[7].

*Results and discussion.* Metaphase chromosomes show deep stained C-bands at telomeric regions of all the chromosomes (figure 1). As there are 16 chromosomes in a cell, 32 bands can be seen in a metaphase plate. At telophase these meta- to submetacentric chromosomes have their telomeres oriented towards the newly formed cell wall (figure 2). Figure 3 presents an interphase cell showing the telophase orientation of the C-bands. Such orientation of the telomeres is also evident in the binucleate cell (figure 4) induced through caffeine treatment (10 mM) and fixed after 20 h at the $G_2$ phase[8] This orientation is also maintained, even in the early prophase cells (figure 5). The observations on the Giemsa C-bands indicate clearly that the telophase arrangement of the chromosomes is maintained throughout the interphase. The highly reiterated DNA in the telomeres may act as anchor regions to the nuclear membrane[6,9] attributing the definite orientation of the interphase chromosomes.

Giemsa C-bands in interphase cells have been studied in rye too[10,11]. In rye they have observed end to end chromosome attachment in interphase cells. In Allium cepa too, 2 by 2 chromosome association has been reported by Fussel[6] by autoradiography. The present observations, however, do not reveal any regular end-to-end attachment of the C-bands. In interphase cells 24–26 bands (instead of usual 32) can be seen (figures 3 and 5). The lower number may be due either to limitation of the procedure or to ectopic pairing between some of the C-band regions as DNA of similar base compositions and sequence might mediate heterochromatic attraction[12,13]. In no case, however, could 2 by 2 pairing be noticed.

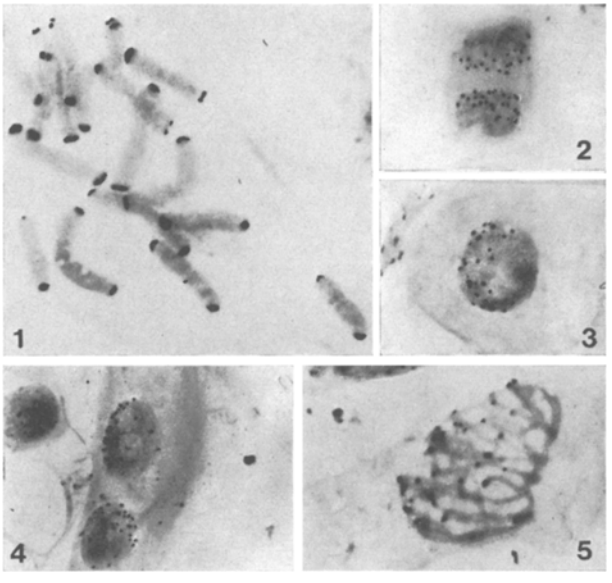


Fig. 1. A metaphase cell of Allium cepa showing 16 chromosomes with C-bands at the telomeric regions. Fig. 2–5. Orientation of C-bands in a telophase cell, or interphase cell, a binucleate $G_2$ cell and an early prophase cell respectively.

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## Effects of $Ag^+$ on frog skin: Interactions with oxytocin, amiloride and ouabain[1]

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*Summary.* Different biological effects of $Ag^+$ ($10^{-4}$ M) were found depending on its presence in the outer or the inner solution bathing the frog skin. A marked increase in the electrical conductance and an interference with the action of oxytocin and amiloride were found only when $Ag^+$ was added to the outer solution. Results suggest that $Ag^+$ affects several transport processes, in particular the permeability of the Na entry pathways.

Sensitivity of amphibian epithelia to the presence of minute quantities of heavy metal ions has been reported[2–7]. In particular, $Ag^+$ induces significant changes in the permeability of frog skin when present in the outer bathing solution[2]. We report here some effects of $Ag^+$ that have hitherto not been described, and observations on its interaction with the biological effects of oxytocin, amiloride and ouabain.

*Materials and methods.* The abdominal skin of frogs Rana ridibunda was mounted on Ussing-type conic chambers

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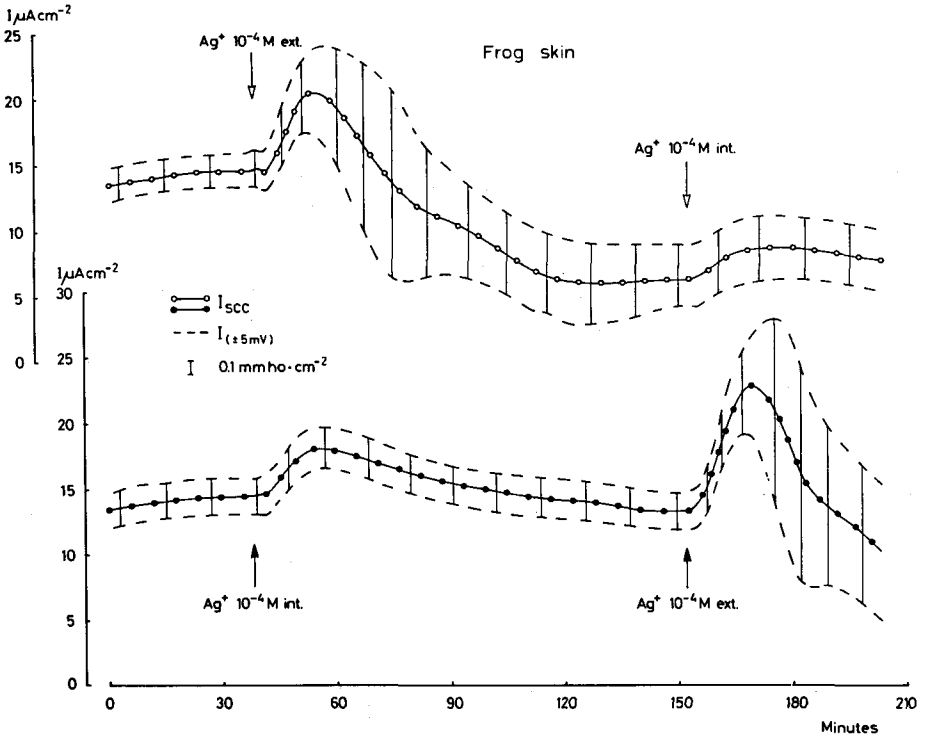


Fig. 1. Time course of changes in SCC and k in paired frog skins after addition of $Ag^+$ to the outer and the inner solution. Note the similarity of the phasic patterns of the responses to $Ag^+$ added to one side in the presence of $Ag^+$ in the opposite side. The continuous lines (●—● and ○—○) represent SCC. The dashed lines (.....) represent the envelope current curves at $\pm$ 5 mV. The vertical bar (I) indicates the total electrical conductance (k) of the skin.

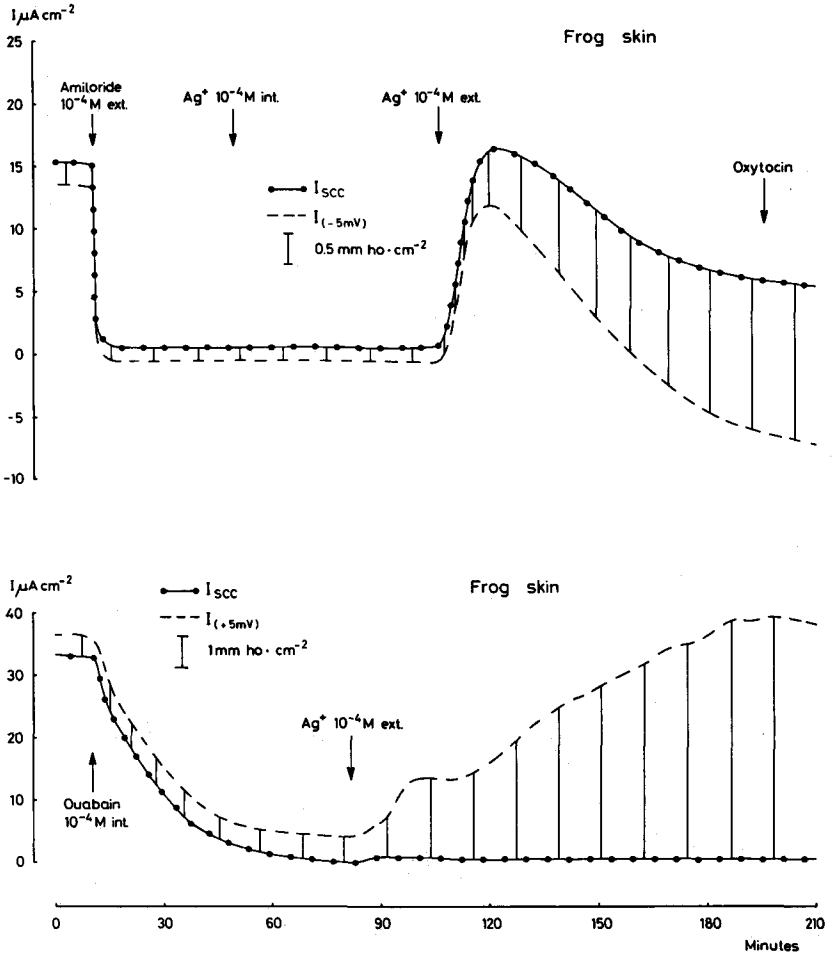


*Fig. 2.* Comparison of the effects of $Ag^+$ in skins pretreated with ouabain (bottom) or amiloride (top). With ouabain, a marked increase in k persisted in the absence of changes in SCC; amiloride blocked the effect of 'internal' $Ag^+$, while 'external' $Ag^+$ induced a remarkable increase in both SCC and k. Symbols as in figure 1.

with an exposed area of 2.27 $cm^2$. The skins were bathed in Na-Ringer solution, the composition of which was (in mM): $Na_2SO_4$, 56; $K_2SO_4$, 1.25; $CaSO_4$, 1; Tris 5 or 10; pH adjusted to 8 with $H_2SO_4$. Aeration and circulation of Ringer solution were accomplished by passing air through a KOH solution and then into the air-lifts. Electrical parameters were measured with routine techniques. A programmable voltage clamp, based on that described by Yonath and Civan[8], was used to control the electrical potential difference (PD) across the skin at 2 predetermined values for variable durations. The electrical currents required to set PD at 0 and 5 mV were monitored throughout the experiment. From the distance between these current envelope curves, the electrical conductance (k) was computed.

*Results and discussion.* Figure 1 illustrates a typical response of the skin to $10^{-4}$ M $Ag^+$. When this cation was added to the *outer* solution, short-circuit current (SCC) fell below the pre-$Ag^+$ level after a transient rise; k increased markedly, but the kinetics of its changes did not follow those of SCC. The concomitant increase in k and decrease in SCC resulted in a precipitous drop in the open-circuit PD. When $Ag^+$ was added to the *inner* solution, although a biphasic change in SCC again occurred, the onset of the response was slower and SCC, in most cases, stayed above the pre-$Ag^+$ level. However, the most distinct characteristic of this response was the lack of change in k during the entire SCC phasic change. The question arises as to the biological significance of the $Ag^+$-induced changes in SCC which has been usually taken as equivalent to net Na transport. To test if this relation still applies in the presence of $Ag^+$, we studied the interaction of $Ag^+$ with three substances having a well documented effect on Na transport across frog skin: oxytocin, ouabain and amiloride.

With $10^{-4}$ M $Ag^+$ in the *outer* solution, a supramaximal dose (40–100 mU/ml) of oxytocin (Syntocinon, Sandoz SA) failed to stimulate SCC in a series of 9 skins. In contrast, in another series of 10 experiments where $Ag^+$ was added to the *inner* solution, the stimulation of SCC by oxytocin was not modified. In fact, initial and delta values of SCC were 14.93 ± 2.49 and 10.64 ± 2.67 μA $cm^{-2}$ in the $Ag^+$-treated skins, while in the control paired skins, the corresponding values were respectively 14.60 + 2.84 and 10.15 ± 1.42 μA $cm^{-2}$. When the sequence of drug administration was reversed, the patterns of response to $Ag^+$ added to the outer or the inner solution of the oxytocin-stimulated skins remained similar to those observed in non-stimulated skins.

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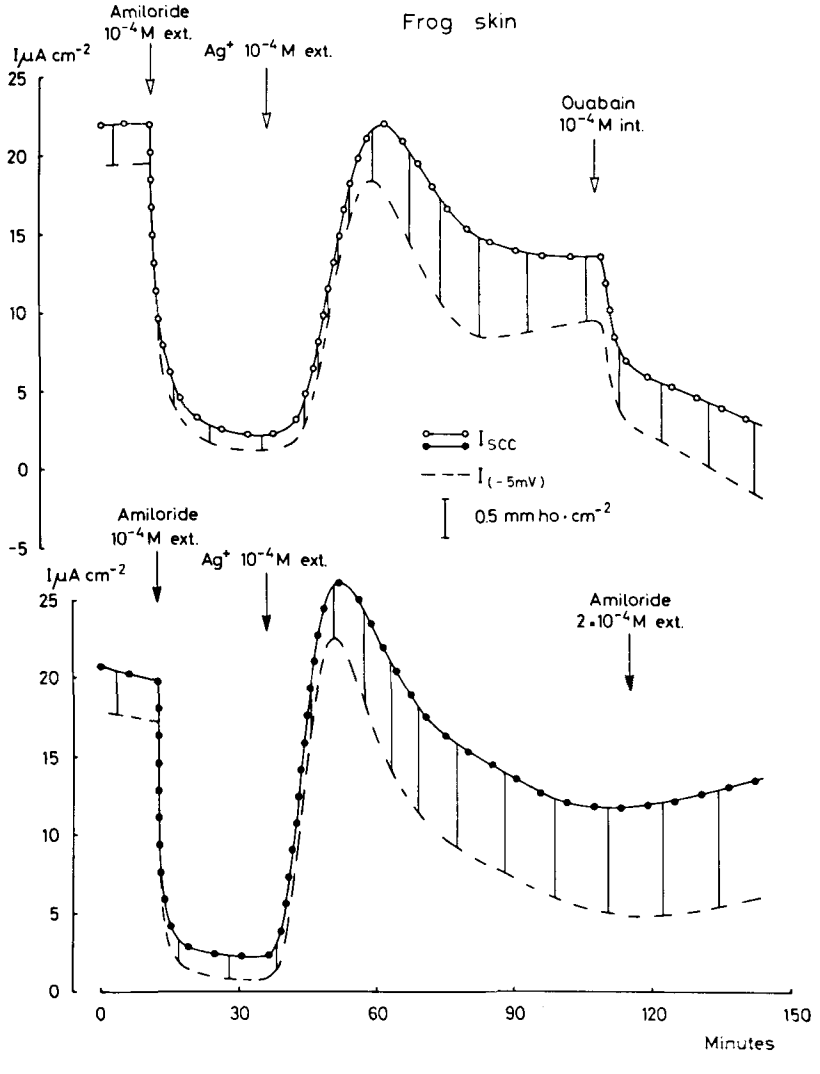


Fig. 3. Comparison of the effects of ouabain and amiloride on the 'renewed' SCC induced by 'external' $Ag^+$. Skins were pretreated with amiloride. Note the sensitivity to ouabain in contrast with the insensitivity to high concentrations of amiloride. Symbols as in figure 1.

Concerning the interaction between $Ag^+$ and ouabain, 2 main features were found as shown in figure 2: a) when the active transport of Na was first abolished by ouabain, $Ag^+$ added to the outer solution could elicit no change in SCC; b) in contrast, a conspicuous increase in k was still observed, indicating a change in passive membrane permeability. Finally, the interaction between $Ag^+$ and amiloride produced the most interesting and unexpected biological effects. Addition of $Ag^+$ to the inner solution of skins pretreated with $10^{-4}$ M amiloride resulted in no change in SCC or k (figure 2). However, when $Ag^+$ was added to the *outer* solution, 2 main phenomena were observed: a) the ineffectiveness of amiloride in skins pretreated with $Ag^+$; b) a 'renewed' SCC in skins pretreated with amiloride.

The first phenomenon was evident even in the presence of very high concentration of amiloride ($\geq 10^{-4}$ M). In a series of 21 experiments, amiloride slightly reduced SCC from an average value of 12.95 $\pm$ 1.29 to 9.88 $\pm$ 0.98 $\mu A\ cm^{-2}$, while in skins non-exposed to $Ag^+$, $10^{-4}$ M amiloride was sufficient to prevent the entry of Na into the epithelial cells and bring SCC nearly to zero. The second phenomenon is illustrated in figures 2 and 3. Although SCC had been almost completely abolished by amiloride, addition of $Ag^+$ to the *outer* solution induced not only an increase in k but also a rapid, phasic and sustained increase in SCC in the direction corresponding to a Na net flux from the outer towards the inner solution. This 'renewed' SCC was neither stimulated by subsequent addition of oxytocin (figure 2) nor inhibited by further addition of amiloride but readily inhibited by ouabain (figure 3).

The effects of $Ag^+$ on frog skin reported here differ markedly from those described with other metal ions[3-5,7,9]. Addition of $Cu^{++}$ and $La^{+++}$ to the inner solution inhibits the natriferic effect of oxytocin[3,9,10]; by contrast, $Ag^+$ blocks the hormonal effect only when present in the outer solution. Moreover, it has also been shown that SCC is still nearly totally inhibitable by amiloride in the presence of $Cd^{++}$, $La^{+++}$ and $Cu^{++}$ in the outer solution[4,9,11]; in contradistinction, $Ag^+$ reduces markedly the sensitivity to amiloride and, most conspicuously, induces a 'renewed' SCC, in skin pretreated with amiloride.

The sum of our results with oxytocin, oubain and amiloride is consistent with the view that changes in SCC induced by $Ag^+$ reflect changes in net Na flux across the skin. The same seems also to apply to the 'renewed' SCC which is inhibitable by ouabain (figure 3). 2 other lines of evidence, recently obtained in our laboratory[11], strongly support this interpretation: first, 'renewed' SCC is also demonstrable when $Li^+$ substitutes for $Na^+$; secondly, in skins pretreated with amiloride, a large increase in the influx of $Na^{22}$ follows the addition of $Ag^+$. However, additional studies are necessary to elucidate the mechanism of the large increase in k observed in different experimental conditions, as well as the chemical species associated with it.

Several possibilities could be considered to explain the observed interaction between $Ag^+$ and amiloride. $Ag^+$ could interact with amiloride receptors of the so-called Na channels of the outward-facing membrane of the skin[12], or open up some amiloride insensitive pathway making Na available to the pump. Being a well-known sulfhydryl reagent, it is likely that the effects of $Ag^+$ on permeability result from an interaction with SH groups of the membrane and/or other cellular components. However, it is noteworthy that other sulfhydryl reagents which we already tested (e.g. PCMBS, methylmercury) have effects quite different from those reported here. At any rate $Ag^+$ appears to be a new and useful tool to help understanding membrane permeability and Na transport at the molecular level.

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## Giemsa band formation in M-chromosomes of Vicia faba[1]

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*Summary.* Various alterations in Giemsa banding technique have been introduced to observe their influence on band formations in M-chromosome of Vicia faba root tip dividing cells. With the introduction of some minor alterations in the technique, revelation of a large number of classes of constitutive heterochromatin has been made possible. Apparently such Giemsa banding pattern is comparable to the ones observed in a routine way amongst mammalian chromosomes.

Although recently various banding methods have been developed for the study of chromosomes, the mechanism of band formation is still a controversy. It has been demonstrated that such banding is not simply the result of denaturation and renaturation of repetitive DNA sequences[3-5], but rather results from modifications of DNA-protein or protein-protein associations[6,7]. The base specific associations which determine Giemsa staining requires an examination of the nature of these proteins to find whether they are acidic and/or basic, as a requisite for evaluating their genetic function. Dick and Johns[8] and Brody[9] have found that acid-alcohol fixation fails to remove quantitatively all the histones from the cytological preparations, while Sumner et al.[10] concluded that histones are mostly removed by fixation. Comings[11] and Comings and Avelino[12] has claimed that band formation can be achieved after histones have been removed from slides by acid extraction. Recently Brown et al.[13] indicate that removal of histones fractions f1 and f2a are necessary for band formation. The present communication deals with the results of Giemsa banding on M-chromosome of Vicia faba after introduction of various procedural alterations in the technique. Growing primary root tips of Broad bean (Vicia faba) has been used in this study. The root tips have been treated with 0.01% colchicine for 2–3 h before fixation in ethanol-acetic acid (3:1) mixture for 8 h. After 8 h they are transferred to ethanol. Squashes of the meristematic root tip cells