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.

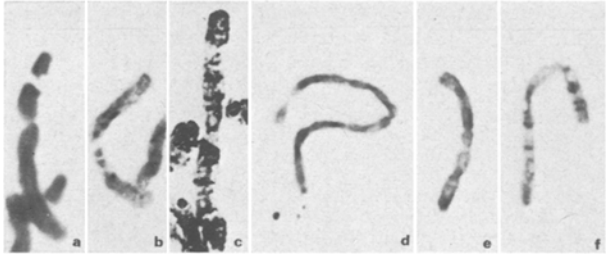
Although recently various banding methods have been developed for the study of chromosomes, the mechanism of band formation is still a controversy. It has been demonstrated that such banding is not simply the result of denaturation and renaturation of repetitive DNA sequences[3-5], but rather results 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

Brief summary of the results of application of Giemsa banding techniques at various altered (in concentration levels of reagents, time and temperature of treatments) treatment conditions

	Pretreatment of the root tips	Treatment	Incubation	Results
a	–	0.07 N NaOH	6 × SSC at 65 °C 12–24 h	Distorted, ghost like swollen chromosomes
b	–	0.07 N NaOH	Sorensens' buffer at 60 °C 12–24 h	Distorted, ghost like swollen chromosomes
c	0.2 N HCl 10-30 min	0.07 N NaOH	2 × SSC or Sorensen's buffer at 60 °C 1-24 h	Distosted, ghost like swollen chromosomes
d	–	Trypsin 0.25% 10–15 sec 25 °C	–	Negative for staining
e	–	5 M or 6 M urea	–	Negative for staining
f	–	10 M $KMnO_4$ with 5 mM $MgSO_4$	–	Positive for staining No differential staining
g	–	2 × SSC at 60 °C 1–24 h	–	Weak staining and no differential staining
h	–	$Ba(OH)_2$	2 × SSC 60 °C 1 h	Positive for centromeric terminal and interstitial heterochromatin
i	–	0.2 N HCl 25–60 °C 10–30 min	2 × SSC 60 °C 1h	Positive for centromeric heterochromatin
j	0.1 or 0.2 N HCl	$Ba(OH)_2$	2 × SSC 60 °C 1 h	Positive for centromeric heterochromatin
k	Pectinas and cellulase with few drops of HCl	$Ba(OH)_2$	2 × SSC 60 °C 1 h	Positive for centromeric and terminal heterochromatin

(fresh or up to 2 days old) have been prepared in 45% acetic acid. Cover glasses are removed immediately after squashing by dry ice method so that the removal of acid-soluble proteins through acetic acid is caused at its minimum. The slides are dehydrated in ethanol for 2–3 h and then air-dried. Freshly made airdried preparations have been used for all the experimental treatments. Various conditions that have been adopted for banding through Giemsa stain, are indicated in the table.

The pertinent features of our observations have been indicated in the table. Use of NaOH with or without other treatments (*a–c* in the table) has caused chromosomal distortion. It has resulted in chromosomes which appear as ghost-like bodies. No banding formation could be achieved by these treatments. Our attempts to cause band formation by the use of proteolytic enzyme trypsin (*d* in the table) and by the protein denaturing substance like urea (*e* in the table) have been unsuccessful. Failure of trypsin treatment to cause bands in air-dried cells without phosphate-buffered Giemsa stain has been experienced earlier[14]. However, Döbel et al.[15] have observed formation of bands in plant chromosomes after urea treatment. The method of Utakoji[16], in which $KMnO_4$ is used (*f* in the table) along with Mg^{++} for band formation, has resulted good chromosome staining, although it failed to produce any longitudinal differential staining (figure, *a*). The procedure in which incubation in 2 × SSC (*g* in the table) has been carried out for 1–24 h, resulted in very feeble or faint staining (figure, *b*). Experimental procedure (*h* in the table) in which there is no HCl treatment or enzyme digestion or alkaline acid treatment, has resulted in causing the formation of both G- and C-bands, i.e. centromeric, terminal and intercalary bands in the chromosomes (figure, *c*). The bands formed near centromere and terminal regions are comparatively darker then those that have been observed in the interstitial regions. It is evident from the figure that at least 16 bands are distributed all through out the M-chromosome.



M-chromosome of Vicia faba after various treatment conditions and Giemsa staining. *a* Method using $KMnO_4$ (details in the table, *f*). *b* Incubation in 2 × SSC (details in the table, *g*). *c* $Ba(OH)_2$ treatment and incubation in 2 × SSC (details in the table *h*). *d* HCl treatment (details in the table, *i*). *e* HCl treatment and incubation in $Ba(OH)_2$ (details in the table, *j*). *f* Enzymes treatment and incubation in $Ba(OH)_2$ (details in the table, *k*).

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Acid treatment and incubation in 2 × SSC with or without $Ba(OH)_2$ (*i* and *j* in the table) have produced some limited band formations. Without $Ba(OH)_2$ treatment (*i* in the table), faint bands have been observed (figure, *d*). In contrast, with the retention of $Ba(OH)_2$ treatment (*j* in the table) dark bands have been obtained (figure, *e*). However, such treatment conditions do not reveal all the bands that have been observed through the method *h*. The use of pectinase and cellulase with few drops of HCl as pretreatment before $Ba(OH)_2$ treatment (*k* in the table), has resulted into appearence of bands in the centromeric and terminal regions of the chromosomes (figure, *f*). Band formations in the interstitial regions of the chromosomes have been extremely poor. Use of the treatment conditions indicated as *h* in the table, has been extended for band formations to somatic chromosomes of Allium sativum and Zea mays. Chromosomes of these plant materials have also exhibited similar banding characteristics.

It is known that the acid-fixed, air-dried chromosomal preparations are left with some histones[9,11]. Treatment with $Ba(OH)_2$ followed with incubation in 2 × SSC at 60°C is considered relatively weak in comparison with NaOH treatment[6] for causing loss of protein or DNA. The treatment conditions containing in method *h* of the table, might have affected superficial DNA-protein associations sufficiently to alter the Giemsa staining reaction, but not significantly to alter the DNA content or packing, as might be expected from vigorous treatments with NaOH, trypsin, urea and $KMnO_4$. It appears that a significant factor which apparently determines band formation is connected with acid treatment. At least, that acid treatment plays a vital role has been documented by Brown et al.[13] in case of G-banding in mammalian chromosomes. Both the methodologies adopted by Schweizer[17] and Gill and Kimber[18] with regard to banding in plant chromosomes contained acid treatment in some or other form. It is known that HCl treatment causes loss of histone from the chromatin material completely[12,19] or differentially for some fractions of histone[9,20]. The characteristic disappearence of G-bands after introduction of HCl treatment in our preparations and the intensity differences observed in the C-bands with and without HCl treatment indicate that histones perhaps play a role in Giemsa band formation.

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Hypophysial hormones and a G_1 block in the lens epithelium of the adult frog (Rana pipiens)

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Summary. Mechanical injury in itself may be responsible for the transition of lens epithelial cells from the G_0 to the G_1 compartment of the cell cycle. This traverse which does not depend on DNA-dependent hypophysial hormones may be in part reversible.

A seasonal variation in mitosis in the anuran lens epithelium has been observed[1,2]. It was suggested that hormones may be influencing this mitotic fluctuation[3]. Hypophysectomy abolishes this seasonal variation in mitosis[4]. Even though injury-induced hyperplasia is characteristic of the lens of the intact frog, mitosis and DNA synthesis fail to occur in response to physical insult in the hypophysectomized frog. Incorporation of ^3H actinomycin D was found to increase in nuclei of lens epithelial cells from hypophysectomized-injured frogs as compared to hypophysectomized-non-injured animals. However, the highest incorporation was found in intact-injured animals. DNA synthesis occurred first near the central mechanical injury in the lens epithelium of hypophysectomized frogs given replacement therapy (hypophysial hormones); but DNA synthesis occurred first in the outer germinative zone in the lens epithelium of similar hypophysectomized frogs given replacement therapy but not given mechanical injuries. It was suggested that lens epithelial cells may proceed through part of the cell cycle without hypophysial hormones[5]. The present report documents further evidence suggesting that mechanical injury by itself may stimulate cells to proceed from G_0 to G_1 and that this transition may be partly reversible.

Materials and methods. Rana pipiens were obtained from Lake Champlain Frog Company, Alburg, Vermont or Ward's Natural Science Establishment, Inc., Rochester, New York. All frogs were maintained in 2 inches of water at 24 ± 2°C. Hypophysectomy was accomplished following a procedure of Hogben[6]. Only the adenohypophysis was removed. Sham operations were performed by drilling through the parasphenoid bone just anterior to the gland. In both cases cerebrospinal fluid was lost. 3 weeks were allotted before experiments were performed to allow levels of hormones to decrease. Mechanical injury was accomplished using a 0-gauge insect pin according to a previously mentioned method[7]. Frogs were anesthesized using Tricaine methanesulfonate (Ayerst Laboratories). The pin was inserted through the cornea and pierced the capsule, epithelium and cortex of the central anterior region of the lens. Lenses were dissected free from the globe and placed in ^3H-thymidine (s.a. 6.4 from New England Nucleur) at a concentration of 5 μCi/ml. The bathing solution consisted of the radioactive label added to Earle's salt solution (Grand Island Biological Company) adjusted to 280 mOsm. Incubation was

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