that chromosome 9 contains a fragile point where the centromeric heterochromatin joins the euchromatin of the long arm[28]. Indeed, cases involving fragility of the heterochromatic segment of chromosome 9 have been reported[30,31]. Obvious length variations are often found in the secondary constriction areas of chromosome 9;[25,27,28,32,33]. Apparently these heteromorphic homologues (Figure 2) segregate normally in pedigrees and should provide valuable data in linkage studies[28].

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We feel that the AS III technique could become a useful procedure for cytogenetic laboratories in the clinical screening of chromosome 9 for possible anomalies. In addition, we hope that the AS III reaction will prove useful to cytochemists in their quest to discover the mechanisms involved in differential staining of human chromosomes.

Résumé. On décrit une nouvelle technique pour les teintures de chromosomes humains. La technique, une modification de l'argent-ammonical, teint sélectivement la région de constriction secondaire du chromosome paire 9, et quelquefois les régions centromériques des chromosomes acrocentriques des groupes D et G. C'est d'un intérêt cytogénétique, puisque les régions chromosomiques sont celles dans lesquelles la fraction satellite DNA III a été découverte par des études d'hybridisation in situ.

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A Pressure Device for Intracellular Injection

An injection device has been developed in which heat is used to elevate the pressure in a micropipette so as to force its content through the tip and into the impaled cell. This technique has the advantage of permitting the injection of both ionic and non-ionic substances and the simultaneous recording of the electrical activity of the cell. Whereas a single pipette suffices for both injecting and recording if the injected fluid is conducting, a double micropipette can be prepared when non-ionic substances are to be injected.

The heating device (Figure 1) consists of a brass tube provided with a rod for fixation to a micromanipulator. The tube accomodates a heating filament (C, Figure 1) supported by a machine screw tightly fitting the upper extremity of the tube. The preparative steps for injection are as follows[1]: a glass micropipette (Corning 7740) partially filled with the solution to be injected (G, Figure 1) is attached to one extremity of a short silver pipe (F, Figure 1) while a piece of the same glass tubing is used to connect the other end of the silver pipe to a drilled machine screw. Solution for injection is then added to the pipette so that it comes into contact with the silver pipe and the connecting glass tube is filled with paraffin oil (E, Figure 1) in order to insulate electrically the pipette from the heating device. The machine screw bearing the mounted pipette is then tightly fitted to the brass tube which was filled before with an excess of alcohol (D, Figure 1). During the assembly of the pipette, care must be taken to avoid formation of air bubbles in any of the liquids used to fill the pipette and the heating device. The entire device is fixed to the micromanipulator and the silver pipe connected to a unity gain electrometer

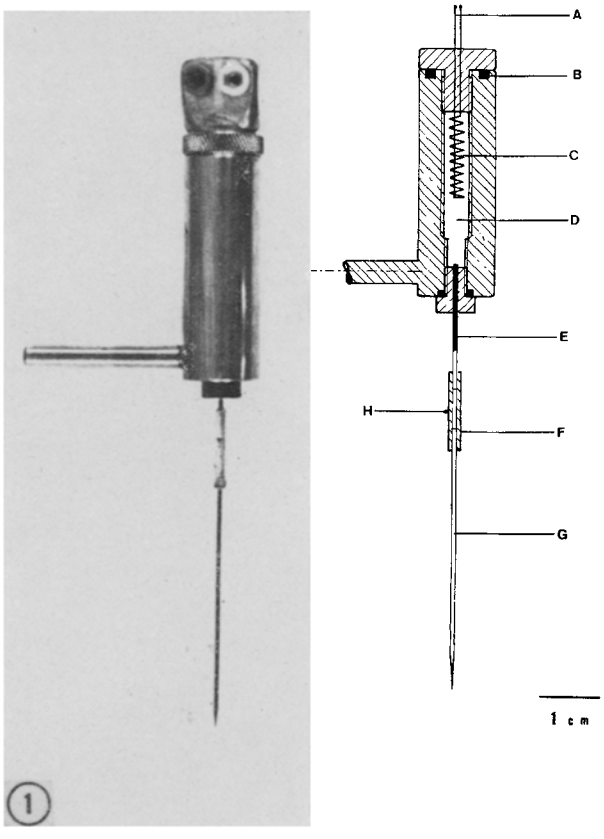


Fig. 1. Pressure device. A) wires connected to the DC current source; B) 0-ring joint; C) heating filament (Ni-Cr-Fe ∅ 0.5 mm 5.7 Ω/m); D) alcohol; E) paraffin oil; F) silver pipe; G) pipette; H) connection for recording.

[1] The mounting of the different elements of the micropipette is carried out with a rapid bounding adhesive (Cyanolit®). This adhesive ensures a leakproof assembly which can be dissolved in acetone and allows the recuperation of the metallic components (silver pipe, machine screw) for making new pipettes.

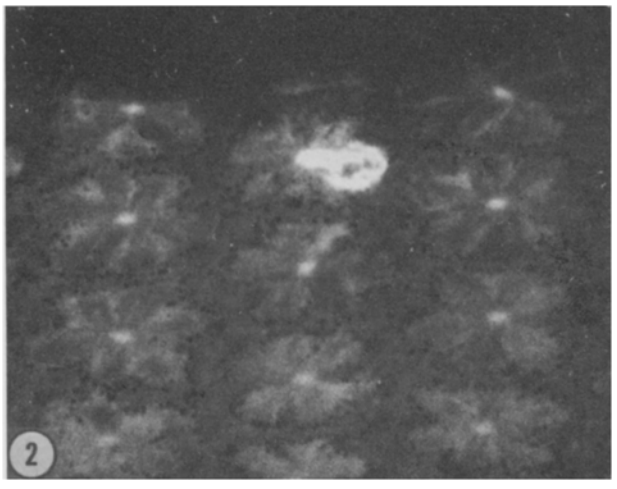


Fig. 2. Transverse section through the retina of the honey bee drone in which a retinula cell was pressure-injected with Procion Yellow. The injected cell, deeply fluorescent, appears in white. Elsewhere, only a weak fluorescence of the rhabdomeres of other ommatidia can be detected. With serial sectioning, the dye was found to fill the whole length of the retinula cell. ×700.

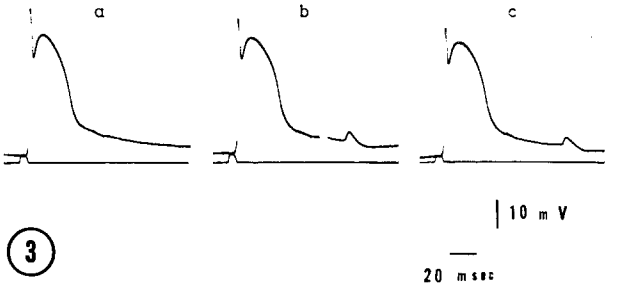
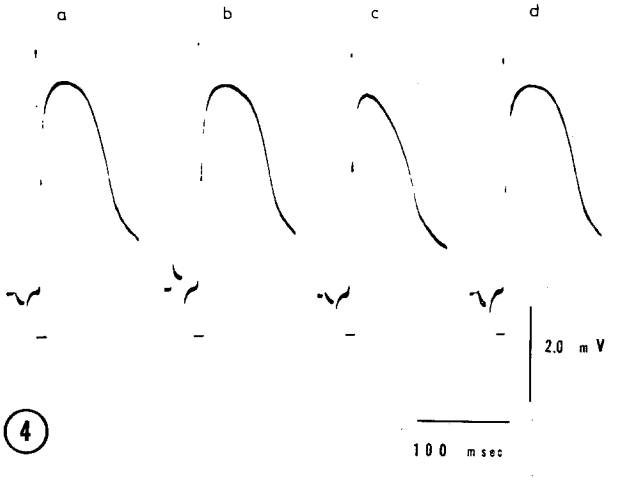


Fig. 3. Responses of the retinula cell shown in Figure 2 to 8 msec flashes during injection. 5 min after impalement of the retinula cell, 4 heating pulses (1 watt, 2 w, 3.2 w, 4.8 w), each lasting 5 min, were given. a) shows the response 5 min after the beginning of the 2 w heating pulse; b) shows the response after 3 min at 3.2 w; c) shows the response at the end of the 3.2 w pulse. Lower trace: light stimulus.



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amplifier. When the wires of the heating filament (A, Figure 1) are connected to a DC current source, the filament heats the alcohol which expands and develops a pressure sufficient to force the solution for injection through the tip of the pipette.

The device has been tested by injecting either dyes or salt solutions into retinula cells of the honey bee drone, while simultaneously recording the membrane potential and the responses to light of the cell. During the injection, the drone retina was maintained in a Lucite chamber, continuously perfused with a *Tris*-Ringer solution, and stimulated with light flashes[2]. Figure 2 shows the result of the pressure injection of a 5% solution of Procion Yellow M4R in a cell of the retina which was subsequently processed and observed in fluorescent light according to Stretton and Kravitz[3]. The responses to light flashes of this cell, recorded at different intervals after the beginning of the injection, are illustrated in Figure 3. Figure 4 shows an injection of 2 *M* NaCl. The effects observed on the amplitude and duration of the response were identical to those obtained by iontophoretic Na^+ injection[4]. The injection of non-ionic substances can be carried out with a double micropipette. One barrel is filled with the solution to be injected and fixed to the heating device in a similar way as for the single pipette. The second barrel, shorter and not connected to the heating device, contains 3 *M* KCl and is used for recording the electrical activity of the injected cell.

Résumé. On décrit un dispositif permettant l'injection intracellulaire de substances ioniques ou non ioniques et l'enregistrement simultané de l'activité électrique cellulaire. Ce dispositif est composé d'un tube de chauffage rempli d'alcool auquel est adapté une micropipette de verre contenant la solution à injecter. Lors du chauffage, la pression causée par la dilatation de l'alcool fait sortir la solution par la pointe de la micropipette. Une double micropipette est utilisée pour l'injection de substances non ioniques.

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Fig. 4. Pressure injection of 2 *M* NaCl in a retinula cell of a drone retina stimulated with 10 msec light flashes at 10 sec intervals. The heating pulse of 1 w lasted 20 sec. Lower trace: light stimulus. Upper trace: responses of the retinula cell. a) control; b) 15 sec after beginning of the heating current pulse; c) 15 sec after the end of the heating current pulse; d) 145 sec after the end of the heating current pulse. In order to test the injection process, negative current pulses are applied through the pipette via a bridge circuit, and precede each response to light. Before injection (a) the bridge is balanced so as to cancel the deflection due to the pipette resistance. When injection takes place, the resistance of the pipette diminishes so that the current pulse is no longer balanced (b). This change in resistance could be explained as follows: the concentrated solution in the tip of the pipette is diluted due to contact with the less concentrated salt solution of the bath and cell cytoplasm. When pressure is applied, an undiluted and thus more conducting solution is forced into the tip and decreases its resistance.

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