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ARE MERKEL CELL–NEURITE RECIPROCAL SYNAPSES INVOLVED IN THE INITIATION OF TACTILE RESPONSES IN SALAMANDER SKIN?

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SUMMARY

1. In salamander skin the Merkel cell–neurite complexes located near the base of the epidermis are the morphological correlates of the rapidly adapting touch receptors (Parducz, Leslie, Cooper, Turner & Diamond, 1977). The present electron microscopic studies revealed that these complexes contain reciprocal synapses polarized in the direction Merkel cell to neurite, and in the opposite direction, neurite to Merkel cell.

2. The possible involvement of chemical transmission in the initiation of the mechanosensory response, was studied *in vitro* with the aid of a stable skin–nerve preparation in which single mechanoreceptors were activated under controlled conditions. Mechanosensitivity was measured with a calibrated prodder (tip diameter 10–30 μm) applied to random or selected points on the surface of the skin while the afferent impulse was recorded in the attached nerve twig.

3. In some experiments the (tungsten) prodder was also used as a surface electrode, allowing the same mechanosensory axon to be excited mechanically (i.e. physiologically), and/or electrically. When applied at a single 'touch spot', suitably timed subthreshold mechanical and subthreshold electrical stimuli could summate to produce a single action potential.

4. The temperature coefficient (Q_{10}) between 5 and 15 °C for the latency of the afferent spike was small, in the range 1·3–2, whether it was evoked by mechanical or electrical stimulation. The latency following the mechanical stimulus, which included the transduction step, was longer than that following the electrical stimulus by 0·5–2·5 ms, and this additional delay was also relatively insensitive to temperature.

5. In several cases removal of the epidermis with its Merkel cells (and presumably the most distal portions of the afferent nerve terminations) did not render the remaining skin totally insensitive to mechanical stimulation; however, the remaining receptive elements, though still rapidly adapting, generally had increased mechanosensory thresholds.

6. The mechanosensitivity of the skin was unaffected by bath application of several aminergic (e.g. noradrenaline, 5-hydroxytryptamine, octopamine) and purinergic (e.g. ATP, quinacrine) compounds at concentrations in the range 0·2–2 mM.

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7. Removal of extracellular Ca^{2+} combined with elevation of extracellular Mg^{2+} (10–40 mM) had relatively little effect on the mechanosensitivity over periods of up to 1 h. In contrast, application of Co^{2+} (2–10 mM) produced a decrease or blockade of the mechanosensitivity that was not associated with any obvious alterations in the ultrastructure of the Merkel cell–neurite complex.

8. These findings do not support the view that conventional chemical transmission (at the Merkel cell–neurite complex) is involved in the initiation of the mechanosensory response. The likeliest conclusion is that the nerve terminal itself is the mechanosensory transducer. Other possible implications of the morphological synaptic relationships are discussed.

INTRODUCTION

The mechanosensitivity of salamander skin is governed largely by the irregular occurrence of discrete, rapidly adapting touch receptors (Cooper & Diamond, 1977). The morphological basis of these mechanoreceptors is the Merkel cell–neurite complex, located near the basal layer of the epidermis (Parducz, Leslie, Cooper, Turner & Diamond, 1977). There is evidence that the Merkel cells in the salamander act as targets for ingrowing mechanosensory nerves (Scott, Cooper & Diamond, 1981) and this also appears to be the case for Merkel cells in *Xenopus* skin (Mearow & Diamond, 1983). The fine structure of the complex in amphibian skin, characterized by the presence of numerous clear vesicles in the sensory nerve endings and large dense-cored granules in the apposed Merkel cell, has led to the suggestion that a reciprocal synapse is present (Fox & Whitear, 1978). However, whether the Merkel cell is actively involved in the mechanosensory function of the receptor complex is presently unclear (Munger, 1977; Diamond, 1979; Gottschaldt & Vahle-Hinz, 1981). Possible physiological roles of the Merkel cell could include modulation of the mechanosensory responses of the sensory nerve endings, or alternatively (e.g. Iggo & Muir, 1969) it may serve as the transducer element itself. The latter, however, has been disputed for the mammalian sinus hair follicles (Gottschaldt & Vahle-Hinz, 1981), where, in contrast to the rapidly adapting character of the complex in salamander (Parducz *et al.* 1977) and *Xenopus* (Mearow & Diamond, 1983) skin, clusters of numerous Merkel cell–neurite complexes function as slowly adapting mechanoreceptors.

In the present study we ask whether the fine structural features of a reciprocal synapse are indeed present at the Merkel cell–neurite complex in salamander skin; in addition a new *in vitro* skin–nerve preparation was developed to study further the physiological characteristics of the mechanosensory complex under controlled conditions. In view of the synaptic features of the association between neurite and Merkel cell that were observed morphologically, it was of particular interest to examine whether chemical transmission was involved in the initiation of the sensory response. Preliminary results of some of these findings have been reported (Nurse, Holmes & Diamond, 1983).

METHODS

Isolated skin preparation

Mature salamanders (*Ambystoma tigrinum*) of length 10–20 cm were usually housed at 12 °C and occasionally at room temperature. Following decerebration of an animal under anaesthesia (0·2 % ethyl *m*-aminobenzoate methane sulphonate (MS 222, Sandoz, Switzerland), in most experiments a patch of skin approximately 10mm × 5 mm with its attached cutaneous nerve branches was excised from the dorsal hind limb. In other experiments a 5–10 mm branch of the cutaneous surae lateralis (c.s.l.) nerve was dissected near its entrance into the skin of the dorsal hind limb and a skin patch with the attached nerve was excised from the anaesthetized animal; the animal was then allowed to recover so that the other hind limb could be used at a later date. The skin–nerve preparation was placed on a flat stainless-steel grid located over a shallow well at the bottom of a perfusion chamber containing a 0·1 % glucose-supplemented (control) amphibian Ringer solution of composition in mM: NaCl, 111; KCl, 1·9; $CaCl_2$, 1·8; $MgSO_4.7H_2O$, 1·2; $NaHCO_3$, 2·4; the pH of control or test solutions was adjusted to *ca.* 7·4 before use. In some experiments the bicarbonate buffer was replaced by 10 mM-HEPES-buffer (Gibco, NY, U.S.A.). When the effects of the divalent cations (Co^{2+} and Mg^{2+}) on mechanosensory function were examined a 200 or 500 mM concentrate of the chloride salt was simply diluted in control Ringer solution to give the required final concentration. The perfusion chamber was fitted with inlet and outlet ports connected to syringes which allowed the solutions in the well to be changed by a push–pull method (Fig. 1). The pores

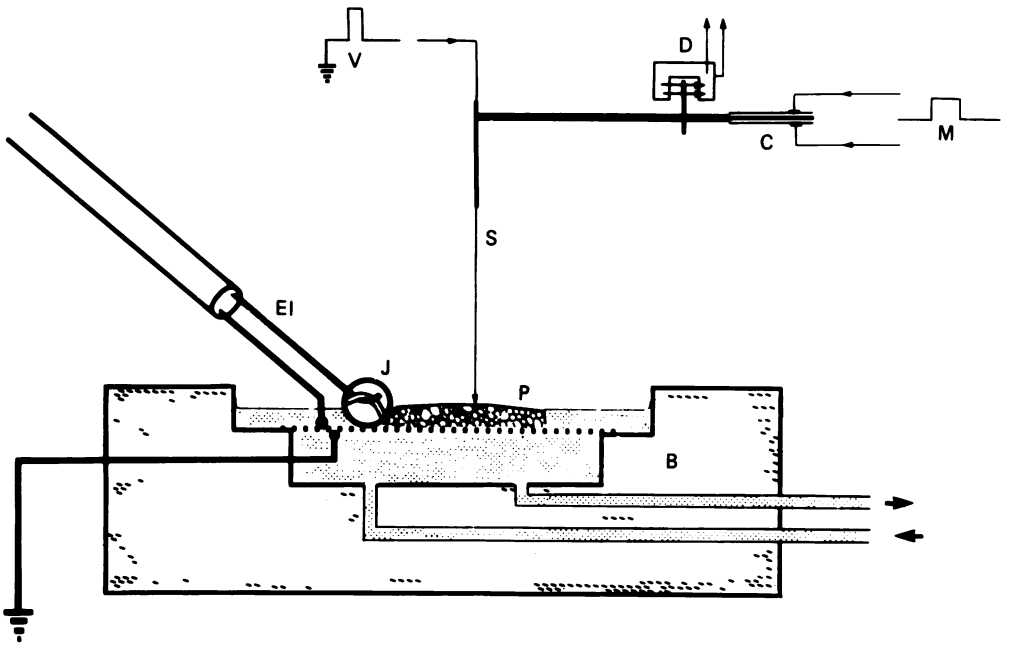

Fig. 1. Diagram of the recording chamber. The bath (B) was made by casting a silicone mould (Sylgard 184, Dow–Corning Corp., MI, U.S.A.) around a 35 mm Petri dish former, and was fitted with a platinum earth, and stainless-steel inlet and outlet tubes (arrow heads). The skin–nerve preparation (P) was supported on a stainless-steel 100 mesh disc, 25 mm diameter, and held in place with 'minuten' insect pins. Impulses were recorded from the nerve twig by a pair of platinum electrodes (El), usually with one of the pair earthed to the steel mesh. The nerve was prevented from drying by application of petroleum jelly (J), while the skin itself was just submerged in Ringer solution. Mechanical (M) and electrical (V) stimuli were delivered to the skin through the same etched probe (S); however, the electrical pulses for each stimulus mode were independent. Movement of the piezoelectric element (C) that drove the prodder (indicated by the small arrow at end of probe) was monitored by an opto-electric detector (D) via a simple bridge circuit.

in the steel grid facilitated exchange of the bathing fluid to the skin, which was usually supported with the dermal side in contact with the grid. After changing solutions the level of the fluid in the chamber was adjusted so that the skin remained just submerged.

Physiology

The isolated skin was mounted in the perfusion chamber and an attached nerve twig was exposed and lifted onto one of a pair of platinum electrodes, while the other electrode was grounded via the steel grid that supported the skin. Afferent impulses were amplified, relayed through an audiomonitor and also displayed on an oscilloscope. An initial test for mechanosensory innervation was made and the receptive field of the nerve twig subsequently mapped, by stimulating the skin with a hand-held bristle and recording the evoked impulse activity in the nerve. For longer term quantitative studies the nerve was sealed around the recording electrode with petroleum jelly to prevent drying. In most experiments the sensitivity and distribution of the mechanoreceptors were determined from an analysis of the (threshold) stimulus intensities required to evoke a single impulse at 50–200 points on the skin with a 10–30 μm tip-diameter mechanical prodder (Cooper & Diamond, 1977); movement of the prodder, which was driven by a piezoelectric element, was recorded with a calibrated photocell device whose output was displayed directly on the oscilloscope. The mechanoreceptors in salamander skin are velocity sensitive, and the velocity of movement of the prodder tip could be varied accurately, and continuously, over a range of 0–35 μm/ms. To test the effect of a drug or of divalent cations the cumulative frequency plots of mechanosensory thresholds over a defined region of skin were compared before, during and after perfusion of the drug or cation using the Kolmogorov–Smirnov test (see Cooper & Diamond, 1977). In addition, the threshold of a selected touch spot was often continually monitored by repeated testing throughout the experiment.

In some experiments the vertical tungsten probe used to prod the skin was modified and used in addition as a stimulating surface electrode. In this modification all but the tapered tip of the probe was sealed in glass tubing while the top of the probe was connected to another stimulator; this allowed the mechanosensory axons to be excited electrically by brief (0·1 ms duration) cathodal current pulses. Since the stimulus to the piezoelectric element of the prodder assembly and that to the tungsten electrode could be applied independently, the mechanosensory axons could be stimulated either mechanically, electrically, or even by simultaneous mechanical and electrical excitation.

Histology and electron microscopy

Pieces of skin were fixed overnight at room temperature by immersion in 0·1-M-phosphate buffer (pH 7·4) containing 2·5% glutaraldehyde and 2% paraformaldehyde; on some occasions 0·5% acrolein was added to the fixative. Samples were then post-fixed in 2% OsO_4 and 1% potassium dichromate, rinsed in sodium acetate and stained *en bloc* with 1% uranyl acetate. Following dehydration in acetone and embedding in Spurr's resin, semi-thin sections were cut and stained with toluidine blue. Transverse ultra-thin sections were grid-stained with 5% uranyl acetate in 50% ethanol and Reynold's lead citrate and were examined for the presence of Merkel cells in a Philips EM 300 electron microscope; for more detailed examination of the fine structural features of the Merkel cell–neurite complex sections were tilted (up to 45 deg) on a eucentric goniometer stage.

In a few studies on 'split-skin preparations' (see Results) transverse sections of fixed skin were cut and examined to test the effectiveness of the procedure used for removing the epidermis from the dermis (see below).

Quinacrine fluorescence of Merkel cells

Among epidermal cells, the Merkel cells in both amphibian and mammalian skin show a rather selective uptake of the fluorescent dye quinacrine (Crowe & Whitear, 1978; Nurse, Mearow, Holmes, Visheau & Diamond, 1983). In the salamander, presumptive Merkel cells were readily visualized in whole mounts of the separated epidermis after intraperitoneal (10–15 mg/kg) or *in vitro* (10^{-6} M for 15 min) administration of the dye (Nurse *et al.* 1983). Separation of the epidermis from the dermis was done mechanically following incubation of whole skin pieces in amphibian Ringer solution containing 1 mg/ml collagenase for 1 h at room temperature, and the quinacrine fluorescent cells were viewed with a Zeiss Universal Microscope equipped with an epi-fluorescence condenser (III RS). The filters used were: exciter BP 436/5, barrier LP 478, beam splitter 460.

Amine fluorescence

To test whether the Merkel cells in salamander skin contain (or can accumulate) any of the common biogenic amines, the glyoxylic acid histochemical technique for whole mount preparations (Furness & Costa, 1975) was applied to the skin. Briefly, pieces of whole skin were treated with collagenase as described above, washed in amphibian Ringer solution and then incubated for 30 min at room temperature in a 2% glyoxylic acid solution in 0·07 M-phosphate buffer; the pH was adjusted to 7·0 with NaOH. The epidermis was then separated from the dermis with forceps, freed of excess moisture and allowed to air dry (basal layer uppermost) on a clean glass slide for 3–5 min. In some experiments the epidermis was separated from the dermis immediately after the collagenase treatment and then incubated for 10–20 min in the glyoxylic acid solution. The slide was then placed in a 90 °C oven for 4–5 min. The tissue was covered with a thin film of paraffin oil, cover-slipped, and then examined for amine fluorescence after exposure to ultra-violet light. To test whether the Merkel cells can take up exogeous amines, whole skin pieces were pre-incubated for 30–45 min in amphibian Ringer solution containing either L-DOPA, noradrenaline (+0·02% ascorbic acid), or 5-hydroxytryptamine at concentrations of 10^{-6} M in the presence or absence of the monoamine oxidase inhibitor pargyline (10^{-4} M); the final pH was adjusted to 7·4. In control experiments the effectiveness of the glyoxylic acid procedure was successfully confirmed on known amine-containing tissues including the salamander pituitary gland and the rat carotid body (J. Diamond, M. Holmes & C. A. Nurse, unpublished observations).

Chemicals

The chemicals used, and their sources were: quinacrine dihydrochloride, aminophylline (BDH Chemicals Ltd., Canada); collagenase (Cat. No.: 840-7018; Gibco, NY, U.S.A.); pargyline; L-β3,4-dihydroxyphenylalanine (L-DOPA), 5-hydroxytryptamine, ATP, caffeine, reserpine (Sigma Chemical Co., MO, U.S.A.). (−)arterenol (noradrenaline: Calbiochem, San Diego, CA, U.S.A.); L-15 growth medium (Gibco, NY, U.S.A.).

RESULTS

Occurrence of reciprocal synapses in Merkel cell–neurite complexes

Morphological evidence for the presence of reciprocal synapses at the Merkel cell–neurite complex in salamander skin was obtained using criteria similar to those discussed by McDonald & Mitchell (1975) for reciprocal synapses in the rat carotid body. At specific junctional regions of the Merkel cell–neurite complex the two apposed membranes (nerve and Merkel cell) were usually distinctly straightened for a distance of a few tenths of a μm, relative to the less regular membrane outlines seen elsewhere (Pl. 1, *A–C*; Pl. 2, *A–D*), and at such regions various 'synaptic' features were observed. A neurite to Merkel cell synapse was characterized by a quite distinct accumulation of clear vesicles within the region of the nerve ending in contact with the Merkel cell, and extending back some tenths of a μm into the terminal; from serial sections these vesicle populations appeared to converge upon sites containing 1–3 approximately triangular 'pre-synaptic' cytoplasmic densities abutting the straightened region of the nerve membrane (Pl. 1*B* and *C*; Pl. 2*B*). Occasionally an omega figure suggestive of vesicle exocytosis was observed at such sites (Pl. 1*B*). In the region of the Merkel cell immediately opposite these presumptive active zones in the nerve ending, there was a relative paucity of dense-cored granules, and (but infrequently) a 'post-synaptic' membrane-thickening or 'fuzz' was observed. The Merkel cell to neurite synapse was characterized by the presence in the Merkel cell of two or more half-moon shaped cytoplasmic densities abutting the cell membrane and one or more adjacent dense-cored granules (Pl. 2*A* and *D*); an occasional omega figure was seen (Pl. 2*C*). On the immediate 'post-synaptic' side there was often an apparent thickening of the nerve membrane and a conspicuous paucity of clear vesicles

in the nerve ending (Pl. 2, *A–D*). In favourable sections Merkel cell to neurite synaptic features occurred side-by-side with those of the neurite to Merkel cell ones, indicating that morphological reciprocal synapses were present (Pl. 1*B*). In other instances serial sections through individual nerve contacts were required to reveal this reciprocity of synaptic relationships (Pl. 2*A* and *B*, Pl. 2*C* and *D*).

Physiological function in the isolated skin preparation

In general a nerve branch could be found in the isolated skin that supplied a defined region whose boundaries could be determined by stroking the skin with a bristle (a crude stimulus) while recording the afferent discharge (e.g. Fig. 4*A*). Spontaneous activity in these nerve twigs was generally rare or absent in normal amphibian Ringer solution, in contrast to whole spinal nerves *in situ*, in which such activity is frequent and arises principally from the non-cutaneous (e.g. muscle, joint) receptors that are excluded from the isolated preparation (Cooper & Diamond, 1977). When recordings are made from the c.s.l. nerve *in situ*, spontaneous activity is also rarely observed, since this nerve may contain cutaneous branches of only the XVIth and XVIIth spinal nerves (Cooper & Diamond, 1977; Scott *et al.*, 1981), and for most of the isolated preparations it is probable that the particular nerve twig investigated was a subdivision of this c.s.l. nerve.

Single mechanoreceptors were activated in the isolated skin by application of the controlled mechanical prodder. Almost all of the mechanoreceptors were rapidly adapting, producing a single impulse irrespective of the magnitude or duration of the stimulus provided this was above the threshold value; this value was in the range of velocities 0·5–2 μm/ms (Figs. 2 and 3*A*). With a just-threshold stimulus the all-or-nothing nature of the afferent spike and the 'jitter' in the spike latency during repeated testing indicated that single units were being activated (e.g. Fig. 3*A*). At room temperature the shortest latencies recorded following suprathreshold stimuli were frequently in the range 2·5–3·5 ms (measured from the initiation of the prodder deflexion until the arrival of the afferent spike at the recording electrode, some 5–10 mm away from the transduction site); since the time-to-peak of the prodder deflexion was usually 2–3 ms, the impulse must be generated early in the rising phase of the mechanical stimulus (cf. Cooper & Diamond, 1977).

Long-term stability of the isolated skin preparation

The rapidly adapting mechanoreceptors in the isolated skin showed remarkable stability as judged by the relative constancy of their thresholds over several hours *in vitro*. For example, in Fig. 2 the thresholds of 100 random points on the skin were sampled within a region *ca.* 3·5 mm diameter at 0, 5 and 22·5 h after skin excision; the 10 μm tip-diameter prodder was moved in steps that varied between 10 and 500 μm. The cumulative frequency plots (Fig. 2) obtained from the histograms of the distribution of mechanosensory thresholds (Cooper & Diamond, 1977) did not change significantly over this period when compared by the Kolmogorov–Smirnov test ($P > 0{\cdot}2$). This preparation was kept at room temperature over the first 5 h, and then at 4 °C until the next sampling session at 22·5 h. Thus after nearly 1 day of separation from the parent ganglion the mechanoreceptors as well as the surviving nerve stump still appeared to function normally. In other preparations mechanosensory thresholds

generally remained quite stable at room temperature over the 6 h period during which most drug experiments (see later) were carried out, but usually gradually declined over the following 20 h. However, in a few cases excised skin was kept at low temperatures (4 °C) for up to 2 days in enriched medium (containing 70 % L-15 growth medium) and the mechanosensitivity was still comparable to that obtained in the freshly excised condition.

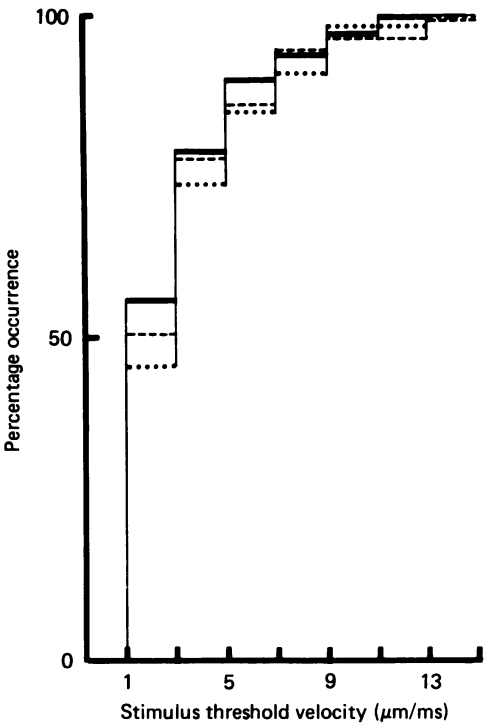

Fig. 2. Stability of touch receptors in the excised skin–nerve preparation. The mechanosensitivity was measured by plotting the percentage occurrence (ordinate) of stimulus threshold values (abscissa) at 100 randomly selected points across the skin. The initial measurements were made immediately after the skin preparation was isolated (continuous line), further measurements were made after 5 h at room temperature (dotted line) and a third time after storage in the refrigerator at 4 °C for a further 17·5 h (dashed line). The three cumulative frequency histograms were not significantly different from each other ($P > 0{\cdot}2$, Kolmogorov–Smirnov test).

Evidence that mechanical stimuli evoked local (electrical) responses

Application of combined subthreshold electrical and mechanical stimuli to a touch spot revealed that their effects were additive when delivered at appropriate intervals. After a 'touch spot' was located, brief (0·1 ms) current pulses of increasing intensity were passed through the dual function stimulator (see Methods) until an afferent spike was recorded. Verification that the mechanically evoked and electrically evoked spikes were carried by the same mechanosensory axon was obtained by an occlusion technique, in which excitation of the axon by one type of stimulation made the axon refractory to excitation by the other; in these cases when the first stimulus was

omitted, the delayed one was effective. In many such situations, one of which is illustrated in Fig. 3, the effects of subthreshold mechanical (Fig. 3*D*) and subthreshold electrical (Fig. 3*E*) stimuli were additive, producing a single mechanosensory impulse if the threshold was exceeded (Fig. 3*F*). These results suggest that mechanical stimulation gave rise to a local electrotonic potential (which in these instances may have been identical to the receptor potential) and furthermore that the subthreshold potentials produced by the electrical stimuli were sufficiently close to the transduction site to allow for spatial and/or temporal summation.

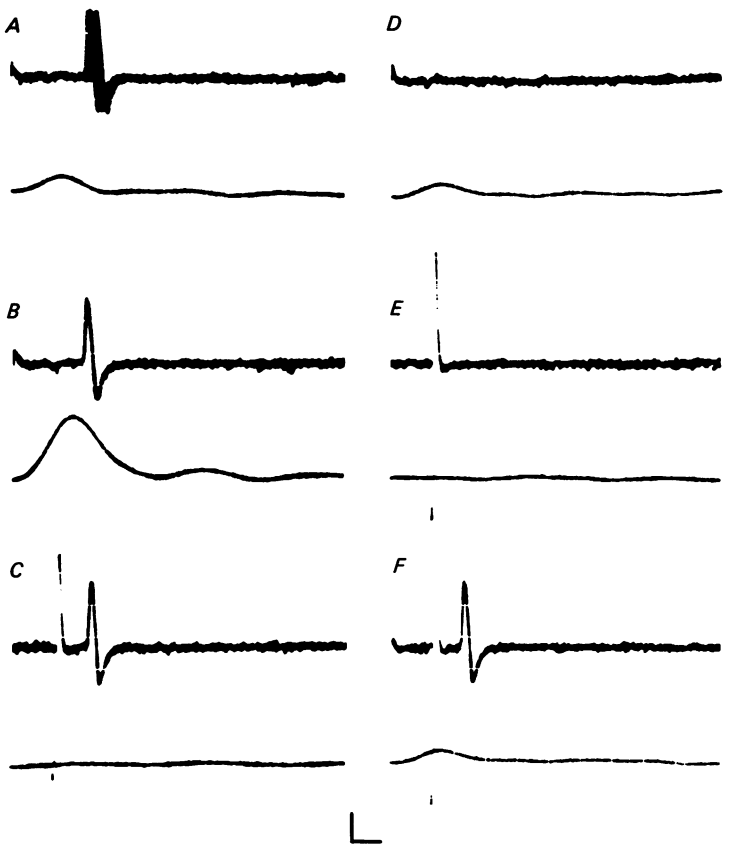

Fig. 3. Interaction between mechanical and electrical stimuli applied to a single touch spot: each pair of traces *A–D* contains seven superimposed oscilloscope sweeps, with the nerve recording shown on the upper trace and the stimulus (mechanical or electrical) to the skin on the lower. *A*, the mechanical stimulus was just at threshold, and there was a characteristic 'jitter' in the afferent spike latency. *B*, the stimulus was increased to a suprathreshold level, producing a constant latency response. *C*, the same mechanosensory axon as that in *A* and *B* was excited by a brief (0·1 ms) current pulse applied via the mechanical (tungsten) prodder; the electrical artifact is seen in the nerve record preceding the impulse. *D* and *E* show the results of applying subthreshold stimuli; *D*, mechanical and *E*, electrical (stimulus artifact only is visible). However, as seen in *F*, when these individually subthreshold stimuli were applied together their effects were additive, resulting in an all-or-none spike in the same mechanosensory axon on each trial. Calibrations: vertical 100 μV (nerve), 10 μm (prodder); horizontal, 2 ms. In all such records the time of initiation of an afferent impulse by the mechanical stimulus is during the rising phase of the recorded prodder movement (Cooper & Diamond, 1977).

Mechanosensory function after removal of the epidermis and its Merkel cells

Adrian and his collaborators showed many years ago that if the epidermis in frog skin was scraped away the tactile discharge in cutaneous nerve fibres can be abolished (Adrian, Cattell & Hoagland, 1931). In the present study, mechanosensory function was tested after a more selective removal of the epidermis and its Merkel cells, achieved by first exposing the isolated skin–nerve preparation to collagenase (1 mg/ml) for 1 h in the recording chamber, followed by mechanical separation of the epidermis (Nurse *et al.* 1983). The enzyme treatment *per se* did not noticeably affect mechanosensory function. After the initial map the epidermis was gently removed and approximately the same area on the remaining skin was remapped. In six out of nine preparations mechanosensitivity was detectable after removal of the epidermis. The cumulative frequency plots of the distribution of mechanosensitivity before and after removal of the epidermis are shown in Fig. 4*D* for one of the successful preparations. Although in these preparations a brisk response could readily be elicited when the epidermis-free skin was stroked with a bristle (Fig. 4*B*; cf. Fig. 4*A*) there was an obvious decrease in the over-all sensitivity, and in addition the mechanosensory thresholds showed a broader distribution (Fig. 4*D*). From the shape and conduction velocity of the recorded afferent spikes it seemed likely that the prodder-evoked sensory responses were carried by identical fibres before and after removal of the epidermis; significantly, the mechanoreceptive elements activated by the prodder after removal of the epidermis also had rapidly adapting characteristics, like their counterparts in normal skin (Fig. 4*C*). In the three unsuccessful preparations mechanosensitivity was abolished since even the large stimulus provided by stroking with a bristle failed to evoke a mechanosensory discharge.

In three of the split skins that were still responsive the separated epidermis was examined for Merkel cells by the quinacrine fluorescence technique (Crowe & Whitear, 1978; Nurse *et al.*, 1983) and their presence in the typical frequency 70–140/mm^2 was confirmed (cf. Scott *et al.*, 1981). An extra indication that Merkel cells were not left behind after removal of the epidermis came from the experiment of Fig. 4, in which frozen sections (20 μm thick) were cut across the physiologically mapped region of remaining skin; examination of these showed that none of the epidermal layers had remained attached to the dermis after mechanical separation. A semi-thin section from a split skin preparation is shown in Pl. 2*E*; the labelling of presumptive Merkel cells by quinacrine fluorescence in the corresponding separated epidermis is shown in Pl. 2*F*. Nerve terminations were likely to be removed with the Merkel cells in these experiments, though this was not studied.

Effects of temperature as evidence for or against the involvement of chemical transmission

Previous studies at the neuromuscular junction indicate that during chemical transmission the synaptic delay and in particular that period associated with transmitter release has a relatively large temperature coefficient ($Q_{10} = 3$; Katz & Miledi, 1965; see also Samojloff, 1925). In the isolated skin lowering the temperature prolonged the latency before the arrival of the mechanically evoked afferent spike at the recording electrode, but the effect was not dramatic (Fig. 5*A* and *B*); in nine tests the average Q_{10} for this delay between 5 and 15 °C was $1{\cdot}42 \pm 0{\cdot}12$ (S.D.; range 1·3–1·7). The Q_{10} was unaffected whether the computations were based on latencies

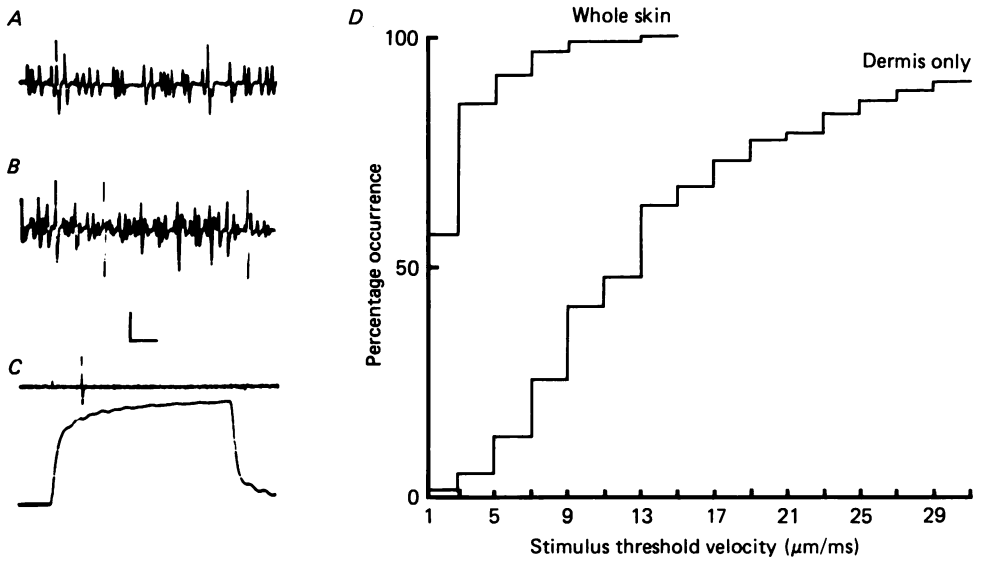

Fig. 4. Altered mechanosensory function after removal of the epidermis. Oscilloscope trace *A* shows the afferent discharge recorded in a nerve twig when a brush was moved gently across a region of epidermis in the attached skin. *B*, a similar stimulus was applied to the remaining (dermal) skin after the epidermis was removed with collagenase. *C* shows the single spike in one axon evoked by a mechanical stimulus delivered by the prodder to the dermal (split skin) preparation; the mechanosensory response (evoked during the rising phase of the stimulus) was typically rapidly adapting, but the threshold was usually larger than normal. *D*, the decrease in over-all mechanosensitivity following removal of the epidermis is shown by the cumulative frequency histograms of mechanosensory thresholds based on a systematic survey of the same region of skin before (195 sample points) and after (188 sample points) removal of the epidermis. The difference between the curves is significant ($P < 0{\cdot}001$; Kolmogorov–Smirnov test: cf. Fig. 2). Calibrations: vertical 100 μV (nerve), 10 μm (prodder); horizontal, 2 ms *A* and *B*, 20 ms *C*.

measured from the initiation of threshold stimuli or of suprathreshold stimuli that were large enough to elicit the shortest latency response at each temperature. In five other preparations use of the dual function stimulator permitted the Q_{10} values to be corrected for conduction time along most of the (myelinated) axonal length. The stimulator was first positioned at a single touch-sensitive spot and Q_{10} measurements for the afferent spike delay were repeated following both mechanical and electrical excitation. By either method the Q_{10} values were comparable (e.g. Fig. 5*C* and *D*) and were similar to those obtained in the above series utilizing only mechanical stimulation (range 1·4–2). Not surprisingly, the Q_{10} for the afferent delay evoked electrically in these experiments was similar to the accepted values for the Q_{10} of axonal conduction in frogs (Q_{10} = 1·5–2; Katz & Miledi, 1965).

Significantly the temperature *vs.* latency curves obtained from the two methods of stimulation were roughly parallel over most of the temperature range (Fig. 5*C* and *D*), and in all cases the latency of the impulse evoked mechanically (measured from the initiation of the prodder deflexion) was longer than that evoked electrically by 0·5–2·5 ms (mean at 20 °C = 1·3 ms; six experiments). This latency difference, due mainly to the time occupied in generating the receptor potential plus that required

to transfer its effects to the impulse trigger zone for the electrical stimulus, was found to be relatively insensitive to temperature; the calculated Q_{10} for the difference between the 'mechanical' and 'electrical' delays in the same axon ranged from 1 to 1·4 between 5 and 15 °C. Thus the mechanically evoked responses are unlikely to depend upon a chemical transmission step.

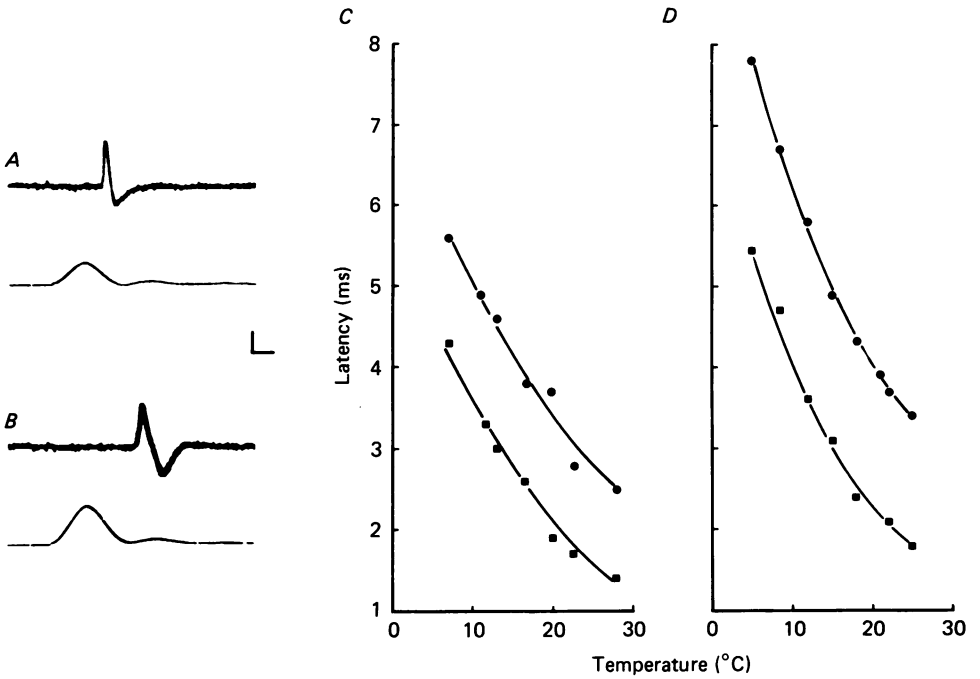

Fig. 5. Effects of temperature on the latency of the recorded afferent spike evoked by both mechanical and electrical stimulation at the same touch spot. *A* and *B* show typical recordings of the shortest latency response (upper trace of each pair) evoked by mechanical stimulation of the same touch spot (lower trace of pair) at 20 °C (*A*) and at 10 °C (*B*); two superimposed traces were recorded for each temperature. *C* and *D* show corresponding latencies of two skin–nerve preparations to mechanical (circles) and electrical (squares) stimuli applied via the dual function stimulator to a touch spot on the skin while the temperature of the bathing fluid was varied. The mechanical delay was measured from the beginning of the prodder deflexion to the initiation of the recorded afferent spike (see *A*, *B*); the electrical delay was measured from the stimulus artifact to the initiation of the recorded spike (see Fig. 3*C*). In these (and similar other experiments) the Q_{10} for both delays varied from 1·4 to 2·0 between 5 and 15 °C. Calibrations: vertical, 50 μV (nerve), 10 μm (prodder); horizontal, 2 ms.

Tests of possible 'purinergic' mechanisms in the mechanosensory response

Uptake of quinacrine by Merkel cells has been taken to indicate the presence of a purinergic function (Crowe & Whitear, 1978; see also Burnstock, 1975). However, several tests failed to implicate this function during mechanoreception. First, neither routine pre-loading of the Merkel cells *in vivo* with quinacrine prior to testing the isolated preparation, nor bath application of the dye (2×10^{-4} M) for periods of up to 1 h to an otherwise normal preparation (five experiments) affected either the mechanosensitivity or the adaptation of the low threshold mechanoreceptors (see

Fig. 6*A*). At the end of such *in vitro* experiments quinacrine fluorescent cells with the expected distribution of Merkel cells (Nurse *et al.* 1983) were present in the isolated epidermis, indicating that the dye had ready access to the Merkel cell–neurite complexes. Secondly, bath application of ATP or adenosine (10^{-4} M) and of the potential purinergic blockers aminophylline or caffeine (Burnstock, 1972) at concentrations of $1–5 \times 10^{-4}$ M (over periods of $\frac{3}{4}$–1 h) has a negligible effect on both the threshold of these mechanoreceptors (Fig. 6*B*) and the receptor adaptation.

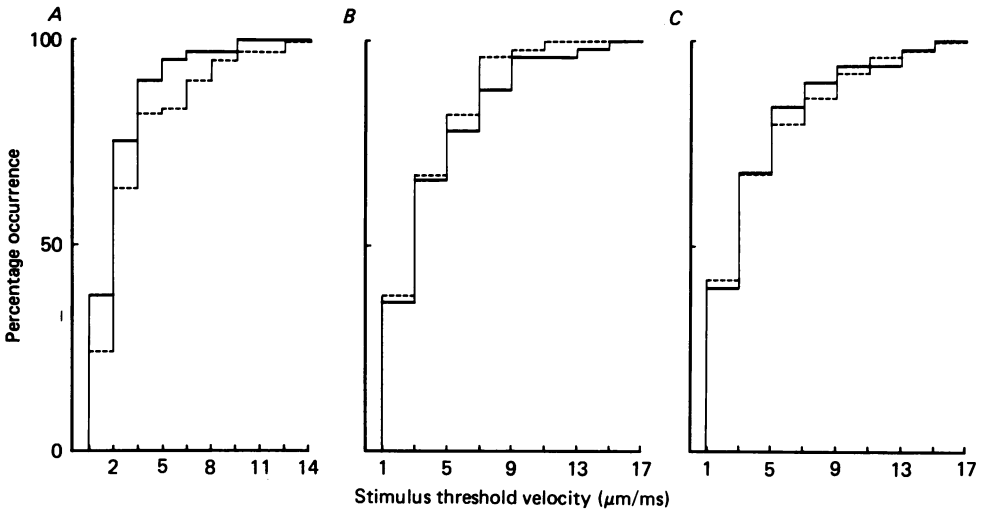

Fig. 6. Failure of purinergic and aminergic compounds to affect the mechanosensitivity of the isolated skin. The mechanosensory thresholds of fifty randomly chosen points were measured over a defined region of skin and the data plotted as cumulative frequency histograms for control (continuous lines) and drug-containing (dotted lines) solutions. *A*, quinacrine dihydrochloride, 10^{-4} M for 45 min; *B*, ATP, 10^{-4} M for 1 h; *C*, noradrenaline, 2×10^{-3} M (+0·02 % ascorbic acid) for 45 min. There is no significant difference between the cumulative frequency plots in control and test solutions for each of the three situations ($P > 0{\cdot}1$, Kolmogorov–Smirnov test).

Do Merkel cells have an aminergic function in salamander skin?

Previous tests using the Falck–Hillarp histofluorescence technique for the detection of monoamines were unsuccessful when applied to the Merkel cells in frog skin (Crowe & Whitear, 1978). In the present study the glyoxylic acid technique for whole mounts (Furness & Costa, 1975) also failed to detect the presence of monoamines in Merkel cells of the separated salamander epidermis. Further, even after pre-incubation of the isolated epidermis (or whole skin) with exogenous amines including L-DOPA, noradrenaline and 5-hydroxytryptamine (10^{-5} M) in the presence of the monoamine oxidase inhibitor pargyline (10^{-4} M), monoamine fluorescence was still undetectable in Merkel cells.

This apparent absence of at least the more common monoamines in Merkel cells of salamander skin paralleled physiological findings that bath application of noradrenaline, 5-hydroxytryptamine or octopamine (10^{-4} to 2×10^{-3} M) for periods of up to 1 h had negligible effect on the mechanosensory response (Fig. 6*C*); cumulative frequency plots of mechanosensory thresholds in the presence of the drug

were not significantly different from controls (seven experiments; $P > 0{\cdot}2$, Kolmogorov–Smirnov test). Further, pre-treatment of salamanders with the monoamine-depleting agent reserpine (20 mg/kg for 2 days) or the inhibitor of 5-hydroxytryptamine synthesis, DL-*p*-chlorophenylalanine (100 mg/kg for 2 days) failed to cause any apparent abnormality in the mechanosensitivity of the skin examined either *in vivo* or *in vitro* (cf. Iggo & Muir, 1969).

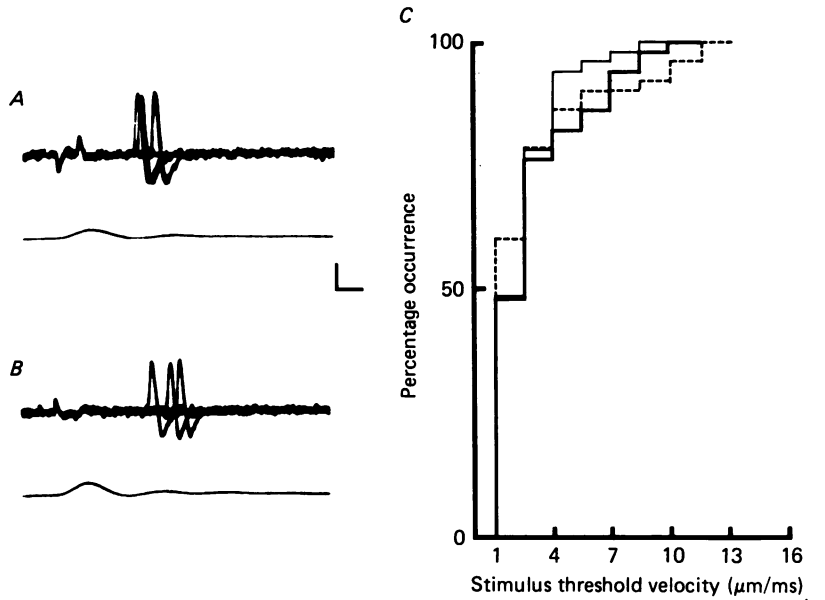

Fig. 7. Negligible effect of Ca^{2+}-free, Mg^{2+}-rich solutions on the mechanosensitivity of the isolated skin. *A* shows the mechanical stimulus at threshold (lower trace) of a touch spot in control Ringer solution containing 1·8 mM-Ca^{2+}, 1·2 mM-Mg^{2+}; nerve responses are shown by the upper trace (six superimposed traces). *B* shows the threshold of the same touch spot after 1 h of perfusion with Ringer solution containing 0 mM-Ca^{2+} and 16 mM-Mg^{2+}. *C*, cumulative frequency histograms based on measurements of mechanosensory thresholds of fifty randomly chosen points over a defined skin region in control Ringer solution (thin line), after 1 h perfusion with 0 mM-Ca^{2+}, 16 mM-Mg^{2+} Ringer solution (thick line) and after washing for 1 h with control Ringer solution (dashed line). There is no significant difference between the curves ($P > 0{\cdot}1$, Kolmogorov–Smirnov test). Calibrations: vertical, 50 μV (nerve), 10 μm (prodder); horizontal, 2 ms.

Tests for chemical transmission by use of divalent cations

A more general test for the occurrence of chemical transmission can be applied by varying the divalent cation concentrations (Katz, 1969). In the experiment shown in Fig. 7, mechanosensory thresholds were obtained from a random survey before, during and after perfusion with Ca^{2+}-free Ringer solution containing 16 mM-Mg^{2+}; the results shown in Fig. 7*C* reveal no significant difference in the mechanosensitivity in control (1·8 mM-Ca^{2+}, 1·5 mM-Mg^{2+}) and test solutions $P > 0{\cdot}1$, Kolmogorov–Smirnov test). Even after 1 h in a low Ca^{2+}, high Mg^{2+} solution touch spots with thresholds in the normal range could still easily be found (Fig. 7*A* and *B*) and there was no evidence for mechanosensory blockade by conduction block or otherwise. In eight similar experiments, removal of Ca^{2+} combined with the addition of

10–40 mM-Mg^{2+} to the bathing fluid had little or no effect on the mechanosensitivity. The rapid effect of Co^{2+} (see below) makes it unlikely that diffusion of the divalent cations was a problem in these experiments.

In contrast to the relative insensitivity of the mechanoreceptors to low Ca^{2+}/high Mg^{2+} solutions, exposure to Co^{2+}-containing solutions was effective; in twenty-five isolated preparations 2–10 mM-Co^{2+} reduced or abolished mechanosensitivity (Fig. 8*A* and *B*). Mechanosensory function was arbitrarily considered abolished when the required threshold stimuli were greater than the mechanical prodder could deliver, i.e. > 30 × normal threshold for touch spots. At the higher doses (8–10 mM) detectable increases in threshold usually occurred within 10 min of changing to Co^{2+}-containing solutions and in most preparations blockage occurred within the next 15 min. In a few cases the effect of Co^{2+} was reversible though it appeared that with more prolonged exposures (> 30 min) recovery was incomplete even up to 2 h after wash-out (Fig. 8*C*).

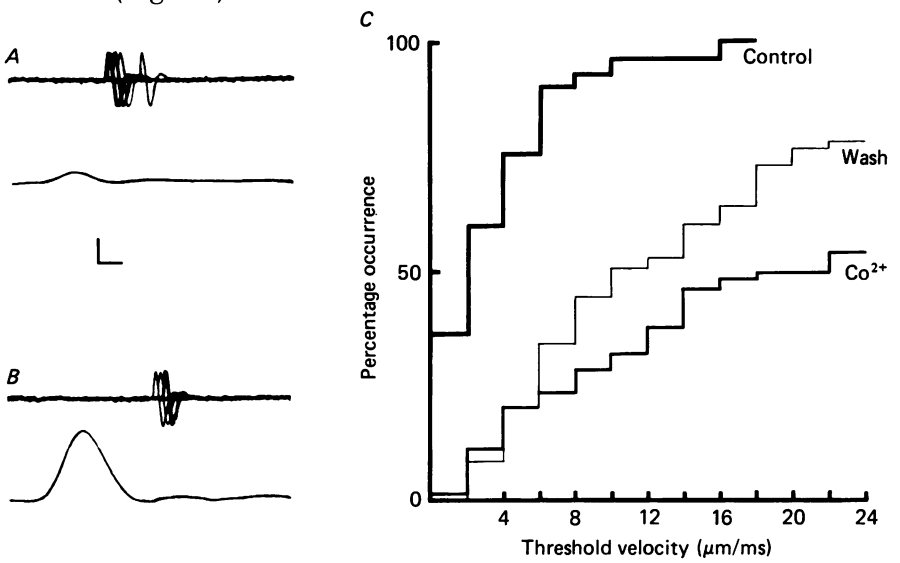

Fig. 8. Effect of Co^{2+}-containing solutions on the mechanosensitivity of the isolated skin. *A*, the mechanical threshold of a touch spot was monitored in control Ringer solution. *B* shows the increased threshold of the same touch spot measured 15 min after perfusion with control Ringer solution containing 5 mM-Co^{2+}. *C*, cumulative frequency histograms based on measurements of mechanosensory thresholds of seventy-five randomly chosen points over a defined skin region in control Ringer solution, after perfusion with 2 mM-Co^{2+} for 40 min, and after a 40 min wash in control Ringer solution. The histograms obtained following perfusion with 2 mM-Co^{2+} and after wash-out are both significantly different from that of the (initial) control ($P < 0.001$, Kolmogorov–Smirnov test). Calibrations: vertical, 100 μV (nerve), 10 μm (prodder); horizontal, 2 ms.

The frequent failure of the mechanosensitivity to recover after exposure to Co^{2+}-containing solutions was not associated with any obvious alteration in the fine structure of the Merkel cell–neurite complex; in preparations blocked with up to 20 mM-Co^{2+} dense-cored granules and clear vesicles were still abundant in Merkel cells and apposed nerve endings respectively although subtle quantitative differences in their number and distribution were not ruled out.

The marked decrease in mechanosensitivity caused by Co^{2+} could also be demonstrated in the split skin (epidermis-free) preparation. In three experiments removal of the epidermis by collagenase caused the increase in mechanosensory thresholds mentioned earlier, but the subsequent addition of Co^{2+} (2–4 mM) caused a further and significant increase in thresholds comparable to that seen in the intact skin preparation. In the absence of Merkel cells (see above) this effect of Co^{2+} must be a direct one on the remaining (mechanosensitive) nerve elements.

DISCUSSION

The present investigations examined the possibility that the synapse-like specializations in the Merkel cell–neurite complexes of salamander epidermis relate directly to the role of these complexes as cutaneous touch receptors (Cooper & Diamond, 1977; Parducz *et al.* 1977). The electron microscopic findings indicate clearly that reciprocal synapses (as defined morphologically) occur between the sensory nerve endings and the Merkel cell. The 'straightened' regions of synapse-like contact between the nerve endings and the Merkel cells are characterized by an increased density of the 'post-synaptic' membrane (especially when this is the nerve membrane), by cytoplasmic densities of characteristic shape abutting the 'presynaptic' membrane and in association with these 'presynaptic' structures the almost invariable occurrence of one or more membrane-bound clear vesicle(s) (within the nerve ending) and dense-cored granule(s) (within the Merkel cell). The reciprocal nature of these synaptic complexes was indicated first by the observation that the morphological polarization of the contact sites could be directed either from neurite to Merkel cell or from Merkel cell to neurite, and secondly by the finding in a number of favourable instances of two oppositely polarized contact sites lying adjacent to one another at the same junctional region. In this initial morphological study these features were not quantified, but the likelihood that they imply a localized release of nerve vesicles and Merkel cell granules is strengthened by the not infrequent observation of omega figures involving the membrane of either the nerve ending or the Merkel cell in close relation to the specific contact sites.

These morphological criteria for the presence of synapses can only be taken as suggestive of, for example, a physiological synaptic function. The fine-structural features of these reciprocal synapses resemble those already reported for frog skin (Fox & Whitear, 1978) as well as for other sensory systems including the olfactory bulb (Rall, Shepherd, Reese & Brightman, 1966) and the carotid body (McDonald & Mitchell, 1975). An initial speculation on a possible function of the reciprocal synapse in the salamander Merkel cell–neurite complex was suggested by a comparison of the mechanoreceptors in amphibian skin, which are rapidly adapting (Cooper & Diamond, 1977; Parducz *et al.*, 1977; Mearow & Diamond, 1983), with the analogous Merkel cell–neurite complexes in mammalian skin. The latter have always been associated with slowly adapting mechanosensory function, e.g. that found for the touch dome (Iggo & Muir, 1969) and the vibrissae (Gottschaldt & Vahle-Hinz, 1981), and the morphological polarization of the synaptic features in these mammalian complexes is usually described as occurring in the orthodromic direction (Merkel cell to neurite; but see Munger, 1977; Mihara, Hashimoto, Ueda & Kumakiri, 1979). Thus

perhaps the operation of an inhibitory feed-back mechanism (via a reciprocal synapse) could serve to transform the type of maintained discharge seen in the mammal to the transient one characteristic of the lower vertebrate.

One implication of this kind of reasoning is that in all species the Merkel cell–neurite synapse is involved initially in evoking impulses in the nerve ending, i.e. that the Merkel cell is the actual transducer. Such a role for the Merkel cell has been disputed in relation to the mammalian sinus hair follicles (Gottschaldt & Vahle-Hinz, 1981; see also Kasprzak, Tapper & Craig, 1970). The present results bear directly on this question, since they fail to implicate the operation of conventional chemical synaptic mechanisms in the initiation of the mechanosensory response at the Merkel cell–neurite complex.

First, in several split skin preparations, elimination of the epidermis with its Merkel cells failed to abolish mechanosensory function in the remaining skin; though the thresholds were increased, the responses were always typically rapidly adapting. This result may imply that the intact Merkel cell–neurite complex facilitates (perhaps mechanically) the delivery of stimuli to the mechanosensitive nerve endings, and/or that the very terminal part of the neurite (in contact with the Merkel cell) is the most sensitive region of a nerve terminal that contains a gradient of mechanosensitivity which increases distally. In this regard it is interesting to note that the axonal terminals in the epidermis (up to, but not including the boutons in contact with the Merkel cell) contain what often appeared to be a layer of mitochondria lining the inner aspect of the axolemma, visible as an outer ring in transverse sections of the axon (Pl. 1 *A*). It is possible that this strategically located cylindrical array of mitochondria is an integral part of the transducer mechanism.

Secondly, the afferent delay associated with the initial phase of the mechanosensory response was attributable to processes that were relatively insensitive to temperature (Q_{10} = 1–1·4) and was therefore unlikely to include chemical transmission, which has a relatively large temperature coefficient (Q_{10} = 3; Katz & Miledi, 1965; see also Samojloff, 1925). This relatively temperature-insensitive delay in the early phase of the mechanical response was in the range 0·5–2·5 ms at 20 °C. That the mechanical stimulus probably gave rise to a local electrotonic potential was indicated by the ability of subthreshold mechanical stimuli to summate with subthreshold electrical stimuli delivered via the same prodder (cf. Gray & Malcolm, 1951).

The electrically evoked impulses in the mechanosensory axons probably originated within their unmyelinated branches. The myelinated portion of the axon begins beneath the dermis, and has a conduction velocity of 5–12 m/s (Cooper & Diamond, 1977); for the 5–7 mm lengths of nerve used in most of the present experiments the corresponding conduction time would be some 0·5–1·5 ms. Since the latency of the electrically evoked spike was typically about 2 ms from the stimulus artifact (Fig. 3*C*) it seems that the extra conduction time of 0·5–1·5 ms must have involved the non-myelinated terminal branches that run within the dermis and basal layers of the epidermis. Assuming a conduction velocity of 0·1–0·3 m/s for these branches (e.g. Katz & Miledi, 1965; Clark, Hayes, Hunt & Roberts, 1984) their lengths would be expected to be in the range of 50–500 μm. These values compare favourably with the size of the mechanosensory axonal fields, whose axial lengths in salamander skin range from 200 to 1500 μm (Cooper & Diamond, 1977).

Thirdly, the effect of Ca^{2+}-removal and/or elevation of extracellular Mg^{2+} (8–40 mM) on the mechanosensitivity was insignificant or only marginal (cf. Clarke *et al.* 1984), although these conditions would be expected to cause a marked depression or blockade of transmitter release (Katz, 1969). The adverse effect on mechanosensitivity of Co^{2+} (2–10 mM) was also exhibited on the split skin (or Merkel cell-free) preparation, implying that it was not dependent on an intact Merkel cell–neurite complex or a functional chemical synapse. Though blockade of mechanosensitivity by Co^{2+} was not readily reversible, there were no obvious alterations in the fine structure of the Merkel cell–neurite junction. While the physiological role of these divalent cations in mechanosensory transduction at the Merkel cell–neurite complex requires further investigations (cf. Akoev, 1982), certainly their effects were not supportive of a chemical transmission link in the initiation of the response in salamander skin.

The pharmacological tests for the possible occurrence of chemical transmission in mechanosensory function focused on the prominent cytoplasmic dense-cored granules of the Merkel cell. The contents of these granules have remained elusive for a long time, and though recent evidence suggests that peptides, particularly Met-enkephalin and vasoactive intestinal polypeptide (VIP), may be present in mammalian Merkel cells (Hartschuh, Weihe, Yanaihara & Reinecke, 1983), physiological roles for these peptides have not yet been demonstrated. In this regard naloxone (10^{-5} M), an antagonist of Met-enkephalin, does not appear to influence the mechanosensitivity of the Merkel cell–neurite complex in the isolated salamander skin (J. Diamond, M. Holmes & C. A. Nurse, unpublished observations; see also Gottschaldt & Vahle-Hinz, 1982). In accord with previous attempts to demonstrate an aminergic function of the dense-cored granules in mammalian and amphibian Merkel cells (e.g. Iggo & Muir, 1969; Crowe & Whitear, 1978), in the present study no demonstrable effects on mechanosensory function were obtained from the use of several monoamines including noradrenaline, 5-hydroxytryptamine and octopamine. Nor did the Merkel cells in salamander skin appear to store monoamines, at least those detectable by the glyoxylic acid histofluorescence technique (see Furness & Costa, 1975). It is noteworthy that the non-biogenic amine quinacrine, a dye that accumulates in Merkel cells and probably within the dense-cored granules (Crowe & Whitear, 1978; Nurse *et al.*, 1983) did not perturb receptor function in the present experiments even at concentrations (10^{-4} M) that inhibit depolarization-induced Ca^{2+} uptake and transmitter release in synaptosomes (Baba, Ohta & Iwata, 1983). Neither does quinacrine uptake by Merkel cells appear to be associated with a 'purinergic' function (Crowe & Whitear, 1978; see also Burnstock, 1975) of the dense-cored granules, since in the isolated skin both ATP and adenosine as well as the inhibitors aminophylline and caffeine had little or no effect on the sensitivity of the mechanoreceptors even at high concentrations (10^{-4} M).

Although a function for the reciprocal synapse at the Merkel cell–neurite complex in salamander skin was not revealed in these experiments, and despite the evidence against the involvement of chemical transmission in the initiation of the mechanosensory impulse, the results do not exclude the possibility that synaptic mechanisms are involved in bringing about subtle modulations of a response generated directly in the nerve ending by the mechanical stimulus. The results, though, do not favour the possibility of a feed-back mechanism that transforms a maintained discharge to

a transient one. Interestingly, in one of the mechanisms proposed for carotid body function, the nerve ending is assumed to be the actual chemosensory element, its activity being subsequently modulated by feed-back from the glomus cells with which the nerve ending has a reciprocal synaptic relationship (McDonald & Mitchell, 1975).

Finally, there are known trophic interactions between the mechanosensory axons and the Merkel cells that act as their targets (see Diamond, 1979; Scott *et al.* 1981; also, Nurse, Macintyre & Diamond, 1984). Recent evidence suggests that the integrity of the Merkel cell–neurite complex may have a role in the development of the characteristic low-threshold mechanosensitivity in *Xenopus* cutaneous axons (Mearow & Diamond, 1983). Conceivably, the chemical signals involved in such trophic interactions are contained within the vesicles of the nerve endings and/or the granules of the Merkel cells. If so, the morphological specializations observed in the electron microscope at the Merkel cell–neurite synapse may well relate to trophic, rather than physiological functions, despite their obvious similarity to those seen at most conventional chemical synapses.

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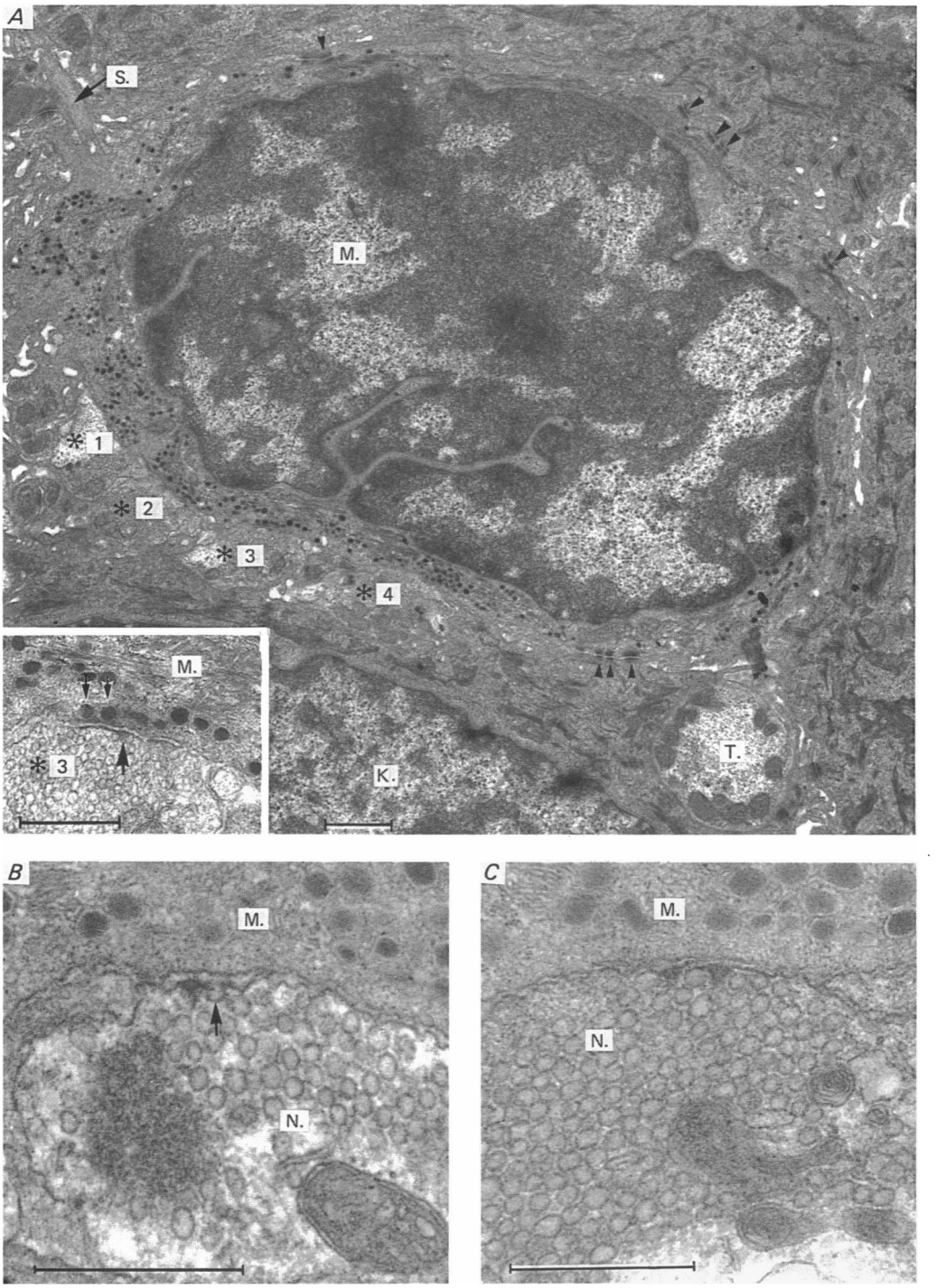
EXPLANATION OF PLATES

PLATE 1

A, salamander Merkel cell (M.) separated from the basal lamina between epidermis and dermis by a keratinocyte of which only a portion is visible (K.). Four nerve endings abut the lower edge of the Merkel cell (*1–4). A profile of an axon terminal (T.) in transverse section contains the layer of dark-staining mitochondria lining the inner aspect of its membrane referred to in the Discussion. The dense-cored granules (d.c.g.s) in the Merkel cell cytoplasm are more concentrated in the general region of the cell contacted by the nerve endings. A typical d.c.g.-free Merkel cell 'spine' (S. and arrow) is visible at the upper left, although this section does not reveal its continuity with the Merkel cell. Note the desmosomal attachments of the Merkel cell to neighbouring keratinocytes (arrow heads). The inset shows a higher magnification of the third nerve ending (N.) in contact with the Merkel cell (M.). There are numerous clear vesicles in the nerve ending, and at least two of these, along with an intervening 'presynaptic' density (large arrow), abut the nerve membrane facing the Merkel cell. To the immediate left of this region, in the Merkel cell, are two d.c.g.s (small arrows) lying in close relation to 'presynaptic' densities that here blend with the cell membrane facing the nerve. Thus some of the features of a reciprocal synapse are present (see Pl. 2). *B*, a nerve ending (N.) in contact with another Merkel cell. To the right of the obvious 'presynaptic' cytoplasmic density an omega figure is seen (arrow). *C*, another Merkel cell–neurite complex, in which two 'presynaptic' densities of characteristic triangular shape are visible in the nerve ending. Calibrations: *A*, bar = 1·0 μm; *A* inset, *B* and *C*, bar = 0·5 μm.

PLATE 2

Reciprocal synapses in Merkel cell–neurite complexes. *A* and *B* show adjacent electron micrograph sections tilted 6 deg and 15 deg respectively in the same direction. *C* and *D* show neighbouring, but not adjacent, electron micrograph sections tilted 12 deg and 18 deg respectively. In the Merkel cell cytoplasm shown in *A* there is a clear concentration of d.c.g.s at a region that contains the typical half-moon-shaped 'presynaptic' cytoplasmic densities abutting the cell membrane (arrows, below which a piece of the section is missing). The apposing membrane of the nerve ending (N.) is thickened, with a paucity of clear vesicles underlying this region of the ending. In the adjacent section (*B*), two presynaptic cytoplasmic densities can be seen to abut the nerve membrane just to one side of the vesicle-sparse region (arrows). Note the absence of d.c.g.s in the region of Merkel cell cytoplasm adjacent to this synaptic zone. Sections *C* and *D* are from the same Merkel cell–neurite complex shown in Pl. 1, *A* (nerve endings 1 and 2). Evidence for the reciprocal synaptic relationship between Merkel cell and nerve can be seen. In *C* some d.c.g.s of the Merkel cell are clustered about an omega figure at the cell membrane (arrow) and opposite this is a thickened nerve membrane, with an underlying absence of clear vesicles. The clear vesicles packed to one side in this profile lie opposite a Merkel cell region devoid of d.c.g.s (*). The region of the omega figure in *C* can be seen in section *D* to contain d.c.g.s in association with densities abutting the Merkel cell membrane. In the right-hand nerve profile in section *C* a triangular 'presynaptic' density is visible (arrow head). *E*, toluidine blue stained semi-thin section of salamander skin containing a region from which the epidermis was mechanically removed after exposure of the skin to collagenase. E., epidermis; D., dermis; B.l., region of basal lamina. *F*, whole mount of collagenase-separated epidermis (from the same skin as in *A*) viewed *en face* after staining for Merkel cells with the fluorescent dye quinacrine. The distribution of labelled cells shown is typical of that of Merkel cells and touch spots in normal (whole) skins (Parducz *et al.* 1977; Scott *et al.* 1981). Calibrations: *A*, *B*, *C* and *D*, bar = 0·5 μm; *E*, bar = 10 μm; *F*, bar = 100 μm.



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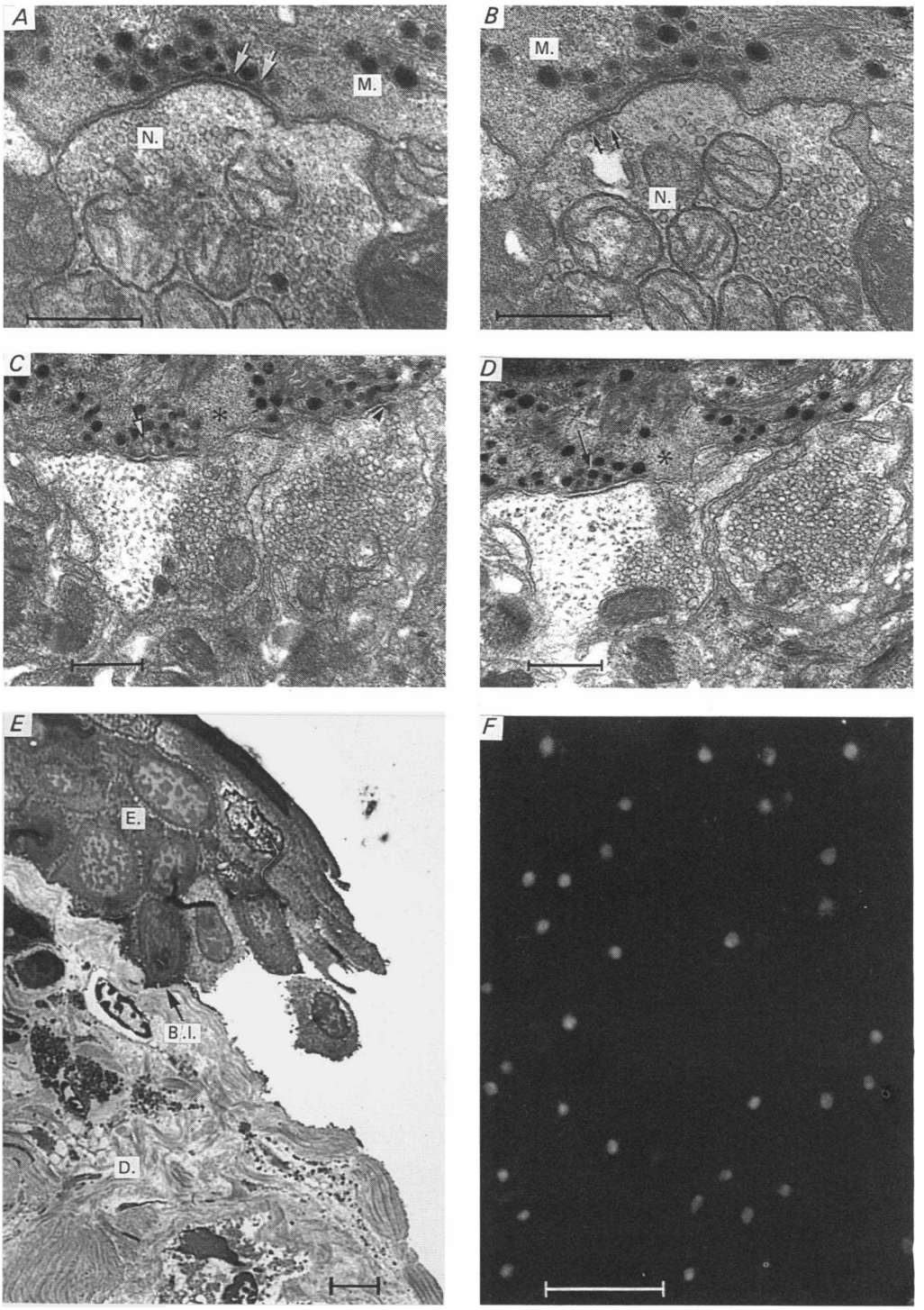

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