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RESPONSIVENESS AND ULTRASTRUCTURE OF SLOWLY ADAPTING TYPE I CUTANEOUS MECHANORECEPTORS IN VITAMIN A DEFICIENT RATS

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SUMMARY

1. Single-unit recordings were made from afferent nerve fibres supplying slowly adapting type I (s.a. I) cutaneous mechanoreceptors in anaesthetized vitamin A deficient and control rats.

2. Trains of thirty repetitive mechanical stimuli with 0·1 s rise time, 1·9 s plateau phase, and 0·7 s interstimulus interval were applied. A feed-back mechanism maintained the force of stimulation at 20 mN during the plateau phases and the contact force between stimuli at 0·5 mN.

3. All displacement values in the group of vitamin A deficient rats were significantly larger than the corresponding control values. Residual indentations were increased by 70–100 % while maximal indentations were only about 40 % higher. These results indicate a non-linear increase in compliance of the skin and underlying tissues.

4. S.a. I receptors were found to be significantly less responsive in vitamin A deficient animals. Mean numbers of impulses were about 25 % lower in the vitamin A deficient group than in controls throughout the entire train of thirty stimuli.

5. In vitamin A deficient rats, Merkel cells and adjoining nerve terminals showed signs of degeneration of a variety of cell organelles, particularly the mitochondria.

6. Degenerative changes induced by vitamin A deficiency especially in the Merkel cells appeared to be a major cause of the reduction of responsiveness in s.a. I receptors.

INTRODUCTION

Vitamin A is a substance required for normal differentiation of epithelial tissues (Wolf, 1984), and its deficiency causes atrophy of the epidermis and its appendages (Wollbach, 1954). Opposing mechanisms have been proposed regarding the effect of vitamin A on keratinization at the cellular level. Fuchs & Green (1981) reported that the production of mRNA for keratin depends on the presence of sufficient amounts of retinoic acid. Conversely cornification in cultured keratinocytes can be inhibited by adding retinoic acid (Yaar, Stanley & Katz, 1981). In chondrocytes the accumulation of fibronectin is enhanced in the presence of retinoic acid (Hassel, Pennypacker,

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Kleinman, Pratt & Yamada, 1979). Fibronectin is a cellular surface protein promoting cohesion between cells. Changes in the pattern of keratinization and cohesion between cells are likely to affect the mechanical properties of a tissue.

The epidermo-dermal border is the site of slowly adapting type I cutaneous mechanoreceptors (s.a. I receptors). Receptors of this type consist of a dome-shaped elevation in the skin containing tactile or Merkel cells in close apposition to terminal enlargements in branches of the afferent nerve fibre (Iggo & Muir, 1969). Nurse & Diamond (1984) found approximately ninety Merkel-type cells per s.a. I receptor in adult rats. However, not all of these cells made contact with nerve terminals. Smith (1967) counted about twenty-five nerve terminals per s.a. I receptor.

The accompanying paper (Baumann, Hamann & Leung, 1986) is concerned with age-induced changes in the force transmission properties of the skin and its effect on the responsiveness of s.a. I receptors. Vitamin A deficiency offers a further model to look at the question of the interrelationship between mechanical properties of the skin including underlying tissues and responsiveness of the receptor.

The responsiveness of a mechanoreceptor is a function of (a) the mechanical properties of the tissue surrounding the receptor (Lindblom, 1965; Hamann, 1980; Pubols, 1982), (b) the normal limits of performance and the condition of the transducer itself and (c) the capacity for nervous conduction in the afferent nerve. It is known that the effectiveness of constant displacement stimuli applied to s.a. I receptors is reduced during vitamin A deficiency (Hamann & Lee, 1982). But it is not known whether this condition affects the relevant mechanical properties of the skin or the transducer structure itself. In the present experiments low force constant force stimulation and electronmicroscopy were employed to provide this information. Some of the present results have been reported as abstracts (Baumann, Cheng-Chew, Hamann & Leung, 1984; Cheng-Chew, Hamann & Leung, 1985).

METHODS

Raising of vitamin A deficient rats

The experiments were performed on Sprague–Dawley rats reared on a vitamin A deficient diet (Purina). Three weeks after birth, the animals were arranged in pairs matching sex and body weight. Each animal was given its own cage. On alternate days the animals were weighed. Rats in the vitamin A deficient group were supplied with diet *ad libitum*. The amount of food consumed and wastage was determined for each rat in this group, and an equal amount of diet was given to the matched control animal the following day. Control animals were given a substitute of 30 i.u. vitamin A concentrate per 40 ml drinking water. As soon as the rate of growth started to slow down in the vitamin A deficient animals, supplements of 7 i.u. vitamin A concentrate were added to each 40 ml drinking water, and if required, higher doses of vitamin A concentrate were given intraorally with a blunted syringe.

Electrophysiological and electronmicroscopical investigations were performed in both groups of animals between weeks 35 and 46 after birth.

Electrophysiology and mechanical stimulation

The rats were anaesthetized with urethane (20 % w/v, 6 ml/kg, I.P.). Supplementary doses were given as required. Single-unit recordings were made from afferent nerve fibres supplying s.a. I receptors in the gluteal region. Nervous responses to stimulation with trains of thirty constant force stimuli were recorded. Individual stimuli rose over 0·1 s to a force of 20 mN at which force level it was kept until the end of the stimulus. Total duration of individual stimuli was 2·0 s. The time between stimuli was 0·7 s. During this time a contact force of 0·5 mN was maintained. Trains of

repetitive stimuli were applied to each receptor between 2 and 5 times. Recovery time between runs was at least 6 min. A detailed description of the method has been given in the accompanying paper (Baumann *et al.* 1986).

Experimental sample for electrophysiology

In six vitamin A deficient rats nineteen units were subjected to a total number of fifty-nine runs in which the force of stimulation was adjusted to 20 mN ($\pm$0·5 mN). The results were compared with those obtained in six control rats with fifteen units and a total number of forty-five runs.

All results are expressed as mean $\pm$ S.E. of mean. For statistical analysis of the data Student's *t* test was used. The minimum requirement for statistical differences was set at a significance level of $P \leqslant 0{\cdot}05$.

Processing of tissue for the electronmicroscope

Skin specimens containing s.a. I receptors were taken from the hind limbs of both control and vitamin A deficient rats and were immersed immediately in a 4 % glutaraldehyde solution (buffered with Millonig buffer, pH 7·3). In order to ensure optimum penetration with fixative and later embedding medium, the specimens were further trimmed into smaller pieces (about 1–2 mm) with a sharp razor blade. After fixation for a period of 4–8 h, the specimens were post-fixed with 2 % osmic acid in Millonig buffer for 2 h. After several changes of Millonig phosphate buffer, dehydration was carried out in a series of up-graded alcohols, and finally the specimens were embedded in resin (Taab, 812). Polymerization was carried out overnight in a Polaron 3 S AL oven set to 60 °C.

The blocks were cut with a Reichert ultramicrotome using glass knives. Thick sections of 1–2 μm were stained with filtered Toluidine Blue solution for localization of Merkel cells. The block was then further trimmed down for thin sectioning. Gold and silver thin sections were cut with a diamond knife (Diatome) and mounted on clean copper grids. Double staining with uranyl acetate and lead citrate was carried out each for 30 min. Stained sections were examined with a Jeol 100 CX 2 electron microscope. A total of twelve receptors from vitamin A deficient and three receptors from control animals were examined. These receptors were obtained from eight different animals (five vitamin A deficient and three controls).

RESULTS

Growth and development

Approximately 9 weeks after birth it became apparent that vitamin A deficient rats grew more slowly than animals in the control group. From this time onwards the general appearance of the animals on a vitamin A deficient diet changed in that the hairs were in an upright position. There was increased loss of hair and the animals started to look unkempt.

Between weeks 10 and 17 many vitamin A deficient rats developed muscular weakness. At the same time the animals ceased to gain weight. The muscular weakness responded well to substitution with small doses of vitamin A concentrate. At week 25, the mean body weights of vitamin A deficient rats were 434·6 $\pm$ 12·5 g ($n = 12$) for male rats and 308·8 $\pm$ 11·3 g ($n = 16$) for females. Both values were significantly lower than those for the pair-fed control groups (male 511·1 $\pm$ 10·1 g ($n = 12$); female 341·1 $\pm$ 10·8 g ($n = 16$); male $P \leqslant 0{\cdot}001$; female $P \leqslant 0{\cdot}01$). It was a common observation during acute experiments that there was little subcutaneous fat in vitamin A deficient as well as in control animals.

During the weeks following cessation of growth, most animals produced a dark discharge from the medial ocular angle, but there was no overt scarring of the cornea. Some rats also developed scoliosis of the spine. From week 37 onwards, loosening and growth abnormalities of the teeth started to become noticeable.

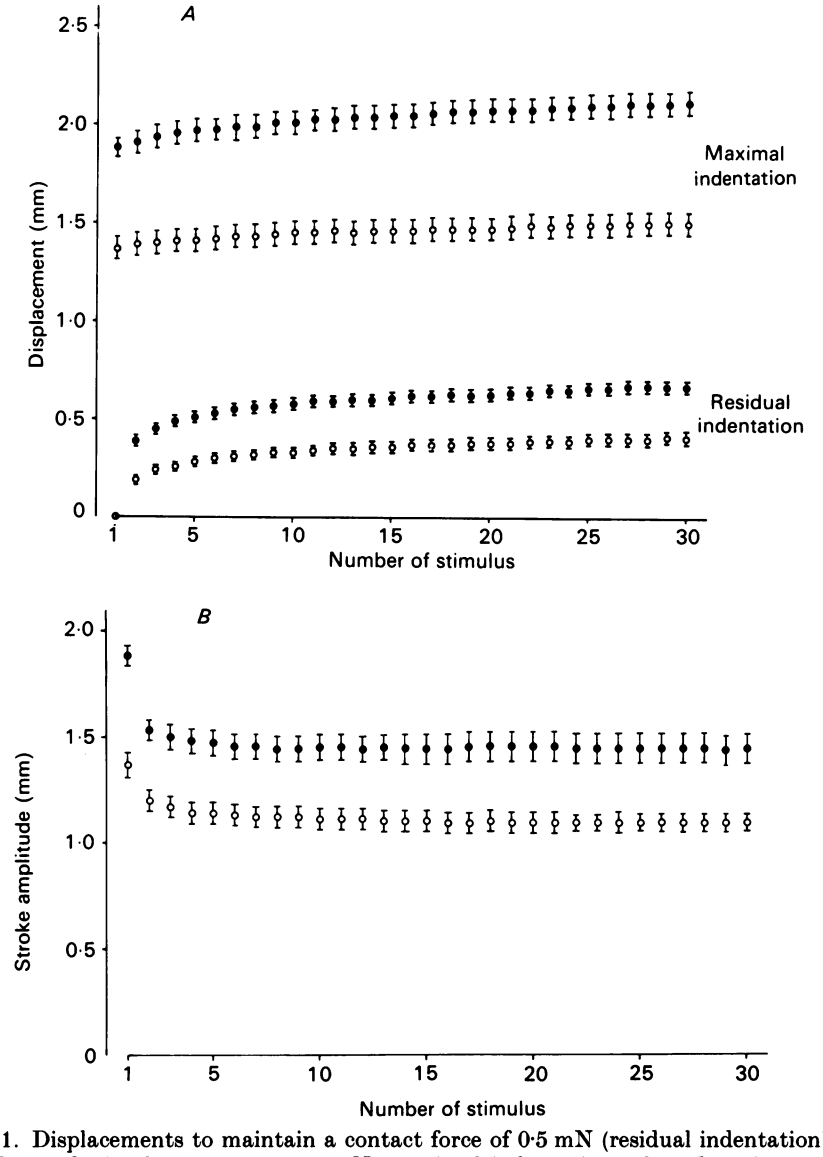

Fig. 1. Displacements to maintain a contact force of 0·5 mN (residual indentation) and the force of stimulation at $20 \pm 0{\cdot}5$ mN (maximal indentation) plotted against ordinal number of stimulus for trains of thirty repetitive stimuli. *A*, residual and maximal indentation. *B*, stroke amplitude (difference between maximal and residual indentation). ●, vitamin A deficient rats; ○, control rats. Comparison of corresponding values between both groups are all statistically significant ($P \leqslant 0{\cdot}001$), except for the residual indentation before stimulus number 1 (zero point).

Displacement during mechanical stimulation

With the type of stimulation employed in the present experiments force was the independent and displacement the dependent variable. A force feed-back amplifier adjusted the displacement to maintain a plateau force of 20 mN during individual stimuli.

The values for maximal indentation during each individual stimulus, residual indentation during the interstimulus period and stroke amplitude (difference between maximal and residual indentation) were all substantially larger ($P \leqslant 0{\cdot}001$) in results from the vitamin A deficient population than in the controls (Fig. 1 *A*, *B*). The mean values from the vitamin A deficient group for maximal indentation were $1{\cdot}88 \pm 0{\cdot}05$ mm at the beginning of a sequence of thirty stimuli and $2{\cdot}12 \pm 0{\cdot}06$ mm for the last stimulus. The corresponding values for the control group were $1{\cdot}37 \pm 0{\cdot}06$ mm and $1{\cdot}50 \pm 0{\cdot}06$ mm. Maximal indentation increased gradually at the same rate between individual stimuli in sequences of stimulation to either group. Throughout the whole train of thirty repetitive stimuli the displacements needed in the vitamin A deficient rats to produce a force of 20 mN were about 40 % larger than the corresponding control values.

After each mechanical stimulus a small dimple was left in the skin. Between stimuli a contact force of 0·5 mN was maintained. Recovery of the skin during this time was slow so that some residual indentation was still present at the beginning of subsequent stimuli. There was substantial residual indentation already just preceding the second stimulus ($0{\cdot}19 \pm 0{\cdot}01$ mm, control; $0{\cdot}38 \pm 0{\cdot}03$ mm, vitamin A deficient). From the second stimulus onwards residual indentation became more pronounced at a gradually decreasing rate with later stimuli in sequences of thirty (Fig. 1 *A*). Residual indentation just before the 30th stimulus was $0{\cdot}41 \pm 0{\cdot}03$ mm for the controls and $0{\cdot}67 \pm 0{\cdot}03$ mm for the vitamin A deficient group. Thus, increase in residual indentation at contact force of 0·5 mN was significantly ($P \leqslant 0{\cdot}001$) larger in the vitamin A deficient animals than in the controls. The difference between residual indentations of the two groups was more pronounced at the beginning of trains of thirty stimuli than at the end.

In the present study stroke amplitude was defined as the difference between maximal indentation during and residual indentation at the beginning of a stimulus. Stroke amplitudes required to maintain a force of stimulation of 20 mN were about 30 % larger in the vitamin A deficient population than in the controls throughout all thirty stimuli. The difference was also highly significant ($P \leqslant 0{\cdot}001$). In both populations there were steep drops between the first and the second stimuli and to a lesser extent between the following four stimuli. Presumably this was due to the extrusion of tissue fluid. Over the following twenty-five stimuli there was relatively little change in the size of the stroke amplitude (Fig. 1 *B*).

Nervous responses

Consecutive absolute mean values of nervous responses decreased over the whole sequence of stimuli in both the control as well as the vitamin A deficient populations (Fig. 2). The drop in responsiveness was most noticeable between the first and second stimuli. The mean response to first stimuli was 206 ± 13 impulses for control units and 155 ± 7 impulses for s.a. I receptors in vitamin A deficient animals. After the first five stimuli mean responses had dropped by one-third, after twenty stimuli by two-thirds and after thirty stimuli by three-quarters of the values of the first responses.

In response to sequences of thirty standard stimuli of 20 mN s.a. I receptors were significantly less responsive in vitamin A deficient rats than in control animals (Fig. 2). The levels of significance for differences between the corresponding means from the two populations were all $P \leqslant 0{\cdot}05$. For nineteen of the thirty responses

the significance level was $P \leqslant 0{\cdot}001$. The responsiveness of receptors in vitamin A deficient rats was reduced by roughly the same extent of 20–30 % of the respective control values throughout the entire train of stimulation.

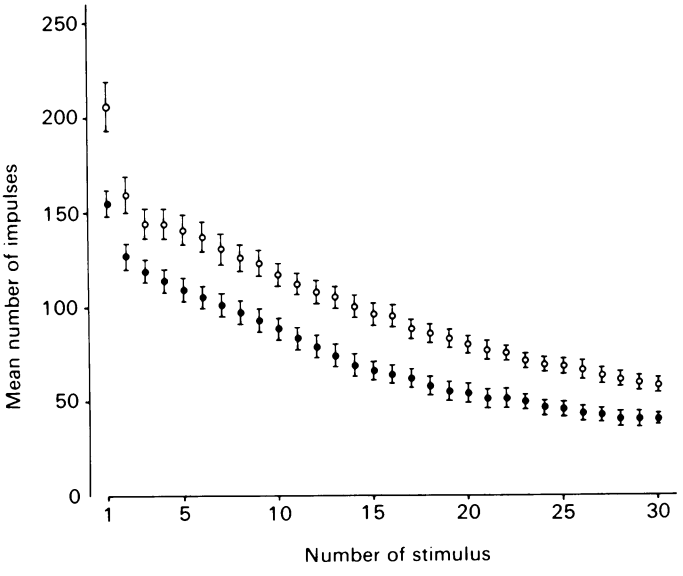

Fig. 2. Responsiveness of s.a. I receptors in mean numbers of nerve impulses per stimulus to trains of constant force stimuli ($20 \pm 0{\cdot}5$ mN) plotted against ordinal number of stimulus. ●, vitamin A deficient rats; ○, control rats. Mean numbers of impulses obtained in vitamin A deficient rats are significantly smaller than the corresponding control values throughout the entire train of thirty stimuli ($P \leqslant 0{\cdot}001$ for stimuli number 2 and number 4–12, $P \leqslant 0{\cdot}05$ for stimulus number 3).

Normal ultrastructure of Merkel cells and nerve terminals

The present observations were in line with reports by other investigators (Iggo & Muir, 1969; Andres & von Duering, 1973; Munger, 1975). Merkel cell nerve terminal complexes were found beyond the basement membrane typically embedded in the basal cell layer (Pl. 1 *A*). Merkel cells contained numerous dense-cored granules preferentially in a position close to the nerve terminal. Their cell nuclei were large and lobulated, and a moderate number of mitochondria were scattered throughout the cytoplasm. Rough endoplasmic reticulum was a common cell organelle. The well-developed Golgi apparatus was usually situated on the opposite side of the Merkel cell neurite terminal. Cytoplasmic spines were another typical feature of the Merkel cells in the present investigation. These spines which were closely related to adjacent keratinocytes did not possess desmosomes, although desmosomes were common structures to be found between these two types of cells in other places.

Ultrastructure of Merkel cells and terminal nerve fibres during vitamin A deficiency

(*1*) *Merkel cell organelles*

(*i*) *Mitochondria.* Swollen mitochondria with lucent matrix and disorganized cristae were frequently observed in Merkel cells from vitamin A deficient rats. Remnants of mitochondrial cristae and thin debris could be seen in some of the swollen mitochondria. Plate 1*B* shows two Merkel cells situated at the epidermo-dermal junction within the basal lamina of the epidermis. The mitochondria impressed by their much larger size compared with mitochondria in Merkel cells from control rats. Membrane destruction seemed to begin with the inner layer, because most of the swollen mitochondria encountered still showed intact outer membranes (Pl. 1*C*). There were hardly any cristae in these mitochondria although some remnants of the inner membranes could still be recognized. The highly swollen mitochondria with disintegrating cristae and lucent matrix made these cells appear much paler than the controls.

(*ii*) *Golgi apparatus and dense-cored granules.* There was moderate swelling of the Golgi apparatus in the Merkel cells from vitamin A deficient rats. Plate 1*D* shows a high-power view of the Golgi apparatus with dilated membranes and vesicles which had not been released into the cytoplasm. Associated dense-cored vesicles presumably packed by the Golgi apparatus also appeared to be dilated.

Although the majority of the dense-cored vesicles in Merkel cells of vitamin A deficient rats appeared normal, dense-cored vesicles with dilated membranes were encountered frequently. The electron-dense material in these dilated granules appeared rather shrunken and irregular as compared to the regular round-shaped normal granules. In some vesicles only a vague dark core could be seen, and some did not contain any electron-dense substance at all (Pl. 2*A*). These unusual vesicles were found largely around the Golgi apparatus. The presence of a moderate number of microtubules (Pl. 2*A*) was also at variance with the controls.

(*iii*) *Endoplasmic reticulum.* Dilatation of endoplasmic reticulum was observed occasionally (Pl. 2*C*). Depending on the degree of dilatation, the affected endoplasmic reticulum had the appearance of vesicles or somewhat larger vacuoles. Because of the attachment of ribosomes to the membranes, dilated endoplasmic reticulum could be distinguished from disintegrated dense-cored vesicles.

(*2*) *Degeneration of Merkel cells*

A few Merkel cells in s.a. I receptors from vitamin A deficient rats were found to undergo terminal degenerative changes. In these cells the nucleus appeared contracted and the cytoplasm was dense. The Merkel cell in Pl. 2*B* is an example for such degeneration. The changes in mitochondria, Golgi apparatus, endoplasmic reticulum and vesicles described above could also be observed. Some of these degenerating Merkel cells showed accumulation of lipid droplets.

(*3*) *Nerve terminals*

The cytoplasm of nerve terminals supplying Merkel cells in vitamin A deficient rats appeared rather clear in comparison with control specimens (Pl. 2*C*). The main observation was an enlargement of the mitochondria similar to what was seen in the

Merkel cells themselves (Pl. 1*B*). The typical cytoplasmic component of the nerve terminal, namely the microfilaments and microtobules did not show up as distinctly as in receptors from control animals (Pl. 2*C*).

(*4*) *Myelinated nerve fibres*

There was no difference between the ultrastructure of nerve fibres encountered in the dermis and cutaneous nerve trunks of receptors from control and vitamin A deficient rats. Specifically, the mitochondria were not enlarged and no abnormalities were observed in the neurofilaments (Pl. 1*E* and *F*). The myelinated as well as non-myelinated fibres of a cutaneous nerve supplying the area of a touch corpuscle under investigation exhibited normal ultrastructure.

DISCUSSION

The present results obtained with constant force stimulation confirmed earlier findings of experiments employing constant displacement (Hamann & Lee, 1982) that the responsiveness of touch corpuscles in rats is reduced during vitamin A deficiency. The mean values for the first response in trains of thirty stimuli were 206 impulses for the control population and 155 for the vitamin A deficient group. The changes in responsiveness were accompanied by increased displacements needed to maintain a constant force of stimulation. Ultrastructurally, vitamin A deficiency induced degenerative changes in the Merkel cells as well as adjacent nerve terminals. The most noticeable features were the enlarged and vacuolized mitochondria.

The force transmission properties of the skin were clearly affected during vitamin A deficiency. Beginning with the first stimulus and for all subsequent stimuli in trains of thirty, a proportionately greater displacement was needed in vitamin A deficient animals to produce the same force of 20 mN than in control rats. Apparently vitamin A deficiency had a softening effect on the skin as tested with perpendicular stimulation. A number of factors could be responsible for this phenomenon. There may be changed water content of the tissue or inhibition of the production of keratin (Fuch & Green, 1981) and fibronectin (Hassel *et al.* 1979).

Although being very pronounced, the changes in the mechanical properties of the skin may be a parallel phenomenon rather than the cause of the altered responsiveness of s.a. I receptors. In the accompanying paper (Baumann *et al.* 1986) conditions were described where large differences in the compliance of the skin have no consistent effect on the responsiveness of s.a. I receptors. Employing trains of constant force stimuli, there were only occasional small differences in the nervous responses for each successive pair of stimuli between populations of young and adult rats although substantially (60–70 %) higher displacements were needed in younger rats to produce the same force. The age-related change in skin compliance was of the same degree at contact force of 0·5 mN and at stimulation force of 20 mN. In contrast the increase in displacements during vitamin A deficiency was more pronounced in the low force range than at 20 mN indicating a non-linear increase in compliance of the skin and underlying tissues.

The present results provide further evidence against an exclusively mechanistic explanation for the reduced responsiveness of s.a. I receptors in vitamin A deficiency.

The same nervous response could be produced by different stimuli in receptors of control and vitamin A deficient rats. For example, to the third stimulus during repetitive stimulation, receptors in vitamin A deficient rats responded with 120 nervous impulses, which is the same as the response to the 10th stimulus in the control animals. Maximal displacements were 1·65 mm for the control population and 1·92 mm for the vitamin A deficient group. Thus, in spite of identical responses, different stimulus parameters were needed.

The ultrastructural results provide good indication for a direct effect of vitamin A deficiency on the sensory receptors. Most prominent were the swollen mitochondria in Merkel cells and in adjoining nerve terminals. The internal membrane structures were broken up in many of the affected mitochondria. Enlarged and distorted mitochondria have also been observed by Leo & Lieber (1983) in hepatocytes of vitamin A deficient rats. The changes observed in this study could be expected to reduce the amount of energy-rich phosphates produced. The present ultrastructural findings match well with the observation of a reduced succinic dehydrogenase activity in the oral mucosa of vitamin A deficient rats (Baume, Franquin & Koerner, 1970). Conversely in the epidermis an increased activity of glucose-6-phosphate dehydrogenase can be observed after application of vitamin A (Jarrett, 1971). The receptor mechanism in touch corpuscles is known to be affected almost immediately by lack of oxygen (Anand, Iggo & Paintal, 1979). Iggo & Findlater (1984) observed a reduction by 90 % in the responsiveness of touch corpuscles in the cat within one minute of recirculating venous blood from the limb under investigation into the arteries and simultaneous replacement of oxygen by nitrogen in the surrounding environment.

It was interesting to note that already beginning with the first response there was a proportionate reduction in the responsiveness of s.a. I receptors in vitamin A deficient rats as compared with the controls, and that it was not progressive. A progressively increasing reduction would have been a more likely time course in a situation of reduced capacity to replenish exhausted energy reserves. Further investigation is needed.

Finally, impaired nervous conduction was probably not the cause for the reduced responsiveness of s.a. I receptors in the vitamin A deficient group. Ceriani, Ventura, Bricca & Rindi (1973) investigated the effects of various degrees of vitamin A deficiency in rats. The vitamin A deficiency in the present experiments was of moderate severity according to their classification. Conduction velocity and refractory period of fast conducting myelinated nerve fibres were not affected by vitamin A deficiency of moderate degrees in their results.

In conclusion, vitamin A deficiency profoundly changes the mechanical properties of the skin as well as the responsiveness of a low threshold epidermal mechanoreceptor, the s.a. I receptor. However, the connexion between these two phenomena may be only coincidental. Pronounced age-related changes in skin compliance had only little effect on the responsiveness of touch corpuscles (Baumann *et al.* 1986). In other studies reduced responsiveness coincided with reduced rather than increased skin compliance for example after chronic treatment with neomycin (Baumann *et al.* 1984) and neonatal treatment with capsaicin (Baumann, Cervero, Hamann & Leung, 1985).

Ultrastructurally, vitamin A deficiency had profound effects on the receptor itself.

Severe degenerative changes particularly in the mitochondria of Merkel cells and nerve terminals were typical for this condition. Chronic vitamin A deficiency produced changes in the mechanical properties of the dermis, epidermis and underlying tissues similar to those observed between young and adult rats. But only vitamin A deficiency affected the responsiveness of s.a. I receptors to a significant extent. Vitamin A deficiency also induced degenerative changes particularly in Merkel cells. Thus reduced responsiveness of s.a. I receptors may have been caused by interference with cellular mechanism rather than by changed force transmission properties.

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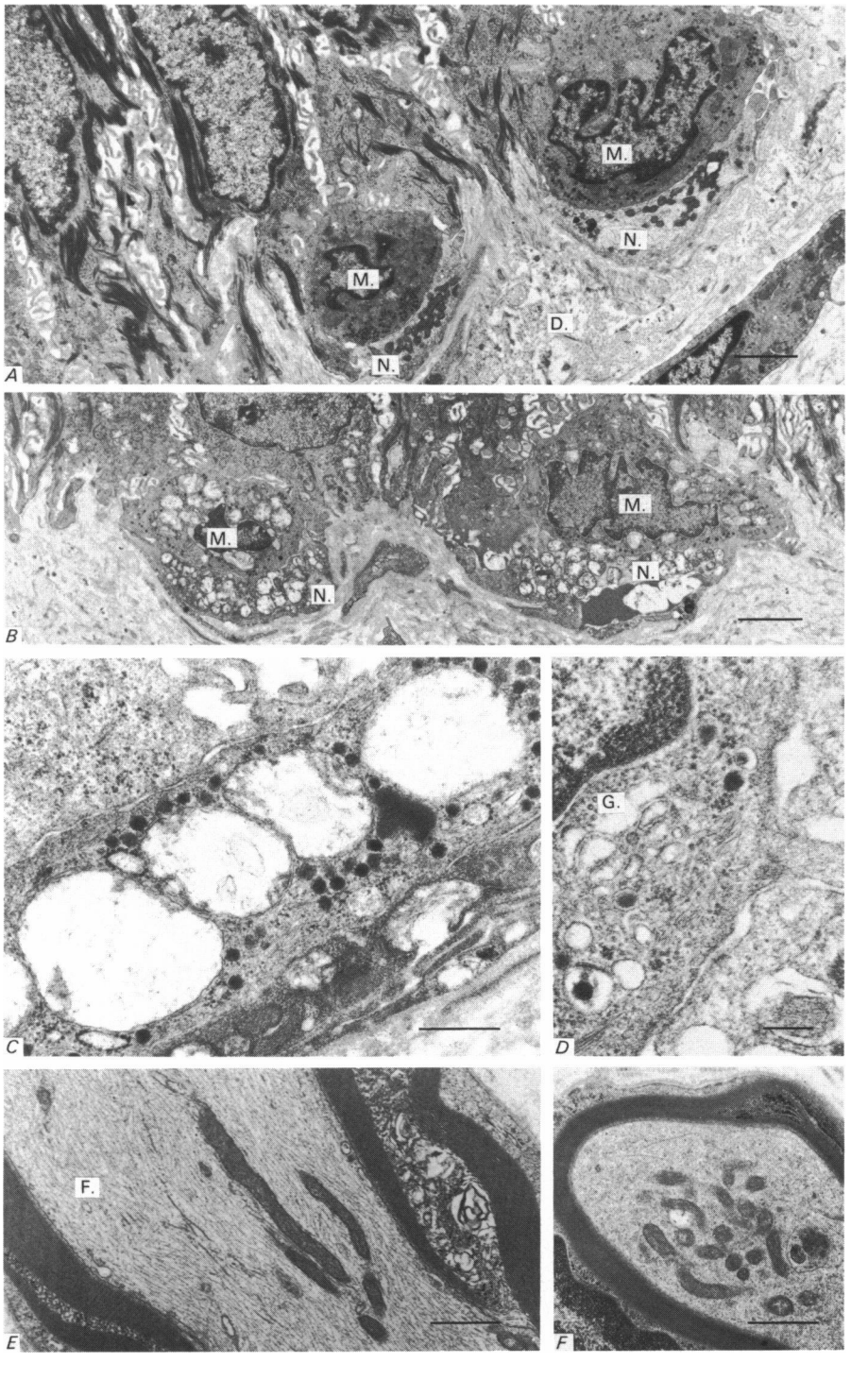
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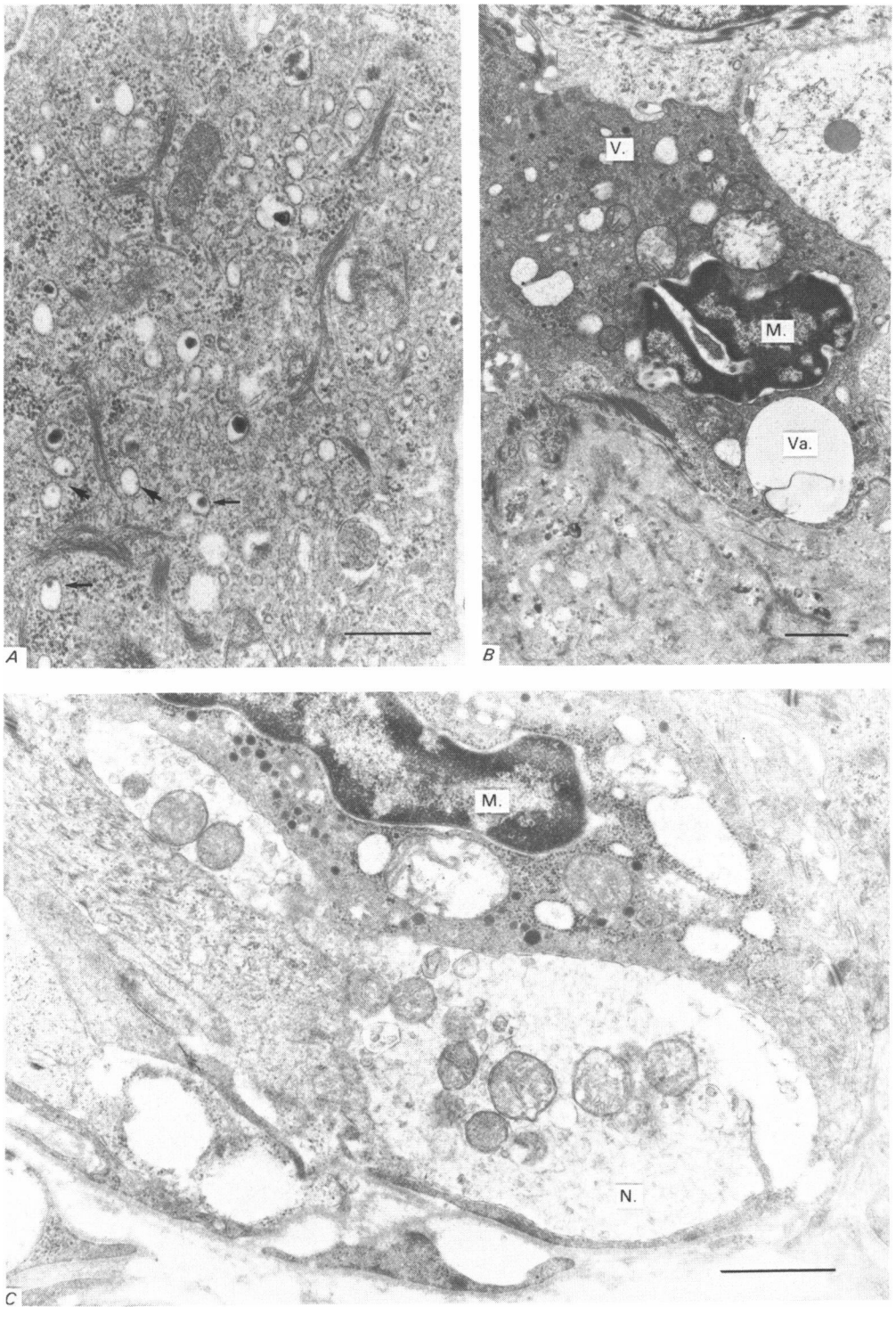
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EXPLANATION OF PLATES

PLATE 1

A, electronmicrograph from a control rat showing a touch corpuscle with two Merkel cells. Note the presence of dense-cored granules and the associated neurite terminals. The Merkel cells are situated at the epidermo-dermal junction. Calibration bar: 2 μm. *B*, a touch corpuscle with two Merkel cells from a vitamin A deficient rat. The mitochondria in the Merkel cells are swollen and disintegrating cristae are present in the lucent matrix. Calibration bar: 2 μm. *C*, a high magnification of a Merkel cell showing swollen mitochondria with lucent matrix and disorganized cristae. Remnants of mitochondrial cristae and their debris can be seen in the matrix of the swollen mitochondria. Calibration bar: 1 μm. *D*, part of a Merkel cell showing moderate swelling of the Golgi apparatus in a vitamin A deficient rat. Calibration bar: 0·5 μm. *E* and *F*, longitudinal and transverse section of dermal myelinated nerve fibres encountered near touch corpuscles in a vitamin A deficient rat. No ultrastructural changes are seen in these fibres. The mitochondria exhibit a normal slender shape. Calibration bar: 1 μm. D., dermis; F., neurofilaments; G., Golgi apparatus; M., Merkel cell; N., neurite terminal.

PLATE 2

A, electronmicrograph of a Merkel cell from a vitamin A deficient rat showing dense-cored granules with dilated membrane. The dense core appears rather shrunken and irregular as compared to the round-shaped normal granules. Some granules have rather vague core material (arrow) and some have even lost their core material and appear like empty vesicles (arrowhead). Calibration bar: 1 μm. *B*, a degenerating Merkel cell from a vitamin A deficient rat. Note the vacuated appearance of the cytoplasm caused by the swollen mitochondria, clear vacuoles and vesicles. The nucleus is contracted and pyknotic. Calibration bar: 1 μm. *C*, a high magnification of part of a Merkel cell and its neurite terminal from a vitamin A deficient rat. The neurite terminal appears rather fluffy instead of filamentous as those observed in the control rat. Calibration bar: 1 μm. M., Merkel cell; N., neurite terminal; V., vesicle; Va., vacuole.