ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. L, 11 SECTIO D 1995

Zakład Stomatologii Zachowawczej. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. Maria Strużak-Wysokińska
Katedra i Zakład Histologii i Embriologii. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. Irena Królikowska-Prasał

Iwona GRAJEWSKA, Irena KRÓLIKOWSKA-PRASAŁ

Ultrastructural Study of Odontoblasts of Dental Pulp in the Cells Aging Process

Badania ultrastrukturalne odontoblastów miazgi zęba w procesie starzenia się komórek

Odontoblasts located in the peripheral layer of the pulp reflect current state of the process connected with the formation of the secondary dentin and thereby they protect teeth from inflammatory processes (1, 3, 4, 6, 10). Odontoblasts are highly specialized cells that can react to the mechanical, thermic and toxic and bacterial traumas (12, 13, 14, 15).

The aim of our experiment was to show the changes of odontoblast epithelium of the pulp in healthy teeth depending on the patient's age. These changes can indicate aging of cells and may enable us to distinguish between physiological changes of the pulp caused by the age of a patient and pathological changes caused by diseases of the teeth.

MATERIAL AND METHODS

The examinations were conducted on 20 healthy teeth, incisors, extracted on medical recommendations. Group I consisted of 10 teeth coming from patients aged between 20 and 30 and group II of 10 teeth — from patients aged betwenn 50 and 60. After longitudinal cut, pulp from teeth was collected. Some of it was preserved in formalin and stained according to the standard method, using haematoxilin and eosine. The remaining part of the pulp was used for ultrastructural examinations conducted by means of transmission electron microscope and scanning microscope. For these examinations the pulp was preserved in 4% solution of glutarate aldehyde in phosphoric buffer with *pH* 7.4 and 1% OsO_4 in phosphoric buffer. For the examinations in transmission electron microscope the material was preserved in Epon 812 resin, in pearls. Sections were cut by means of Reichart's microtome and contrastive staining using uranyl acetate and lead citrate was conducted. Photographs were taken by means of Tesla BS-500 transmission electron microscope.

Some of the material after being preserved in glutarate aldehyde and OsO_4 solution was used for examinations conducted by means of scanning electron microscope. For this purpose, the preparations were dehydrated in a series of alcohols of increasing concentration, dried in 40°C in liquid carbon dioxide of 60 Atm pressure. Dried preparations were sprinkled with gold. Vacuum

sublimation was conducted using Tesla Uz-80 apparatus. Photographs of the preparations were taken by means of Tesla BS-301 scanning electron microscope.

RESULTS

Experimental Group I

In the experimental group I i.e. in teeth coming from patients aged 20—30, the darkly coloured layer of odontoblasts composed of several series of cells was visible (Fig. 1). Beneath, a light cell-free layer (the zone of Weil) and a specific pulp with numerous cells and vessels were observed. Odontoblasts were located adjacent to one another, with their nuclei big and darkly coloured. On the surface of the cells from the dentinal side processes entering dentin tubules were visible.

The structure of odontoblast, in which big, oval nuclei were distinguished, was observed in the electron microscope pictures of the pulp. In the nuclei there were chromatin granules, accumulated mainly in cell membrane's area (Fig. 2). In the nucleus areola pores were visible. Intracellular organelles: endoplasmic reticulum, mitochondria, Golgi complex and single vacuoles were present in the cytoplasm. Near odontoblasts, nerves with dark myelin sheath forming sub-odontoblastic plexus were observed. Figure 3 shows an odontoblast with a nucleus containing chromatin granules and a nerve with clearly coloured myelin sheath. Near the nerve there are a number of colagen filaments.

In the specific pulp there were spindle-shaped fibroblasts with elongated nuclei in which nucleoli could be seen (Fig. 4).

Examination in the scanning electron microscope of the dental pulp showed the basic structure of odontoblastic zone (Fig. 5). Collagen fibers in the pulp were numerous, but lacked any particular orientation in most areas.

Experimental Group II

In the experimental group II, that is in the pulp of teeth coming from patients aged 50—60, some changes in the histological pictures of the pulp in comparison to the pulp of younger patients (of the experimental group I) were observed. The changes pertained to the odontoblasts layer which was thin and irregular and its cells formed usually 2 strata (Fig. 6).

Ultrastructural examinations of odontoblasts indicated diminishing and flattening of the cells. The nuclei were also smaller and shrunk (Fig. 7). In the nuclei chromatin granules accumulated both in the cell membrane area and in the midportion of nucleus were observed. Mitochondria were swollen with slightly marked cristas. In some odontoblasts vacuolation of cytoplasm was observed.

Near the odontoblasts nerves and collagen fibers were visible. Figure 8 presents an odontoblast with a nucleus containing chromatin granules. Next to it there are 2 nerves with intensively stained myelinic sheath. In the specific pulp fibroblasts and macrophages were visible (Fig. 9). In the pulp aging process we observed reduction in the number of cells especially of the odontoblasts and partly fibroblasts. It can be estimated approximately that the number of cells was reduced by half.

Odonotoblasts' layer seen in the scanning electron microscope also indicated a reduction in the number of cells in odontoblastic epithelium (Fig. 10).

DISCUSSION

Physiological processes related to aging of pulp are often similar to pathological changes caused by inflammatory and other diseases (2, 5, 11, 15). That is why ultrastructural observations of the cells are useful, as they make it possible to define functional state of the pulp. The examinations of pulp cells in healthy teeth of patients of various age may reflect the changes taking place in them. Many works concerning pathological changes in teeth e.g. related to caries, paradentosis and others, have confirmed significant degenerative changes in the pulp, related to its fibrosis, numerous inflamatory infiltrations and change in its structure. This is very often accompanied by necrotic focuses and pulp calcification depending on the course of inflammatory process (1, 7, 8).

Our examinations concerned evaluation of ultrastructural pictures of healthy pulp in young patients aged 20—30 and older ones aged 50—60, which means that they were connected with the pulp aging processes. Special attention was paid to odontoblasts which as highly specialised cells are able to produce basal substance of the dentin and influence upon its mineralization (3, 11). Irritations of odontoblasts may cause changes in the pulp and dentin because of the formation of sclerotic dentin resulting from closing of dentinal tubules. Therefore morphological changes in the odontoblasts may reflect changes taking place in both tissues i.e. in the pulp and dentin. The comparison between the morphological changes of the odontoblasts in the pulp of young people and these of the older ones may be of significant importance for the understanding of the whole odontoblast process (4, 9, 12).

Examinations of the pulp in case of caries and paradontosis show that in the case of a far-gone disease changes in the histological picture of the pulp appear (3, 8). That is why ultrastructural changes in the pulp cells were studied, that are connected with the aging processes and may be of extreme significance in the evaluation of the development of a disease as well as its influence upon the pulp. Such changes may be visible only in the cell's ultrastructure, in its nucleus and cytoplasm. Examinations in the light microscope are not sufficient and cannot provide a definite answer.

It has been confirmed that with teeth of people aged 20—30 regular pictures of the pulp are observed. The odontoblasts are cells with big, oval nuclei and numerous cytoplasmic organelles. However in the case of older people, aged 50—60, although the teeth had no lesions, changes in the odontoblast layer, reduction in the number of cells, vacuolisation of cytoplasm and changes in mitochondria and nucleus chromatin were observed.

Conclusions

1. Changes in the histological and ultrastructural pictures of the pulp are connected with aging processes of the cells.
2. The changes included reduction in the number of odontoblast, their vacuolisation, athropy of mitochondria's cristas and nucleus chromatin.
3. The observed changes of the pulp reflect the activated state of its cells, especially of the odontoblasts.

REFERENCES

1. Barańska-Gachowska M. et al.: Rozpoznanie kliniczne a obraz histopatologiczny miazgi zębowej. Czas. Stomat. **21**, 991, 1968.
2. Bernick S., Nedelman C.: Effect of aging on the human pulp. J. Endod. **1**, 89, 1975.
3. Grajewska I., Strużak-Wysokińska M.: Badania morfologiczne miazgi zęba w chorobach przyzębia. Czas. Stomat. **46**, 227, 1993.
4. Григорян А. С. et al.: Ультраструктурная организация одонтобластив в различных фазах функциональной активности. Стоматология **4**, 1, 1979.
5. Jańczuk Z.: Zapobieganie i leczenie chorób przyzębia. PZWL, Warszawa 1992.
6. Królikowska-Prasał I., Czerny K., Majewska T.: Histomorfologia narządu zębowego. Wydawn. Delfin, Lublin 1993.
7. Kurol J., Magnusson B. C.: Intraocclusion of primary molars, a histologic study. Scand. J. Dent. Res. **92**, 564, 1984.
8. Lopez N. J. et al.: Histological differences between teeth adult periodontitas and prepubertal periodontitis. J. Periodontol. **61**, 87, 1990.
9. Morse D. R.: Age-related changes of the dental pulp complex and their relationship to systemic aging. Oral. Surg., Oral Med., Oral Pathol. **72**, 721, 1991.
10. Negm M. et al.: Clinical and histological study of human pulpal response to new cemente containing calxium hydroxide. Oral Surg., Oral Med., Oral Pathol. **50**, 462, 1980.
11. Nielsen C. J. et al.: Age-related change in reducible crosslinks of human dental pulp collagen. Arch. Oral Biol. **28**, 759, 1983.
12. Roth G. I., Calmes R.: Oral Biology. The C. V. Mosby Company, St. Louis—Toronto—London 1981.
13. Smith H. S., Soni N. N.: Histologic study of pulp capping in rat molars using Calcitonin. Oral Surg., Oral Med., Oral Pathol. **53**, 311, 1982.
14. Walts A., Paterson R. C.: A comparison of pulp responses to different materials in the dog and the rat. Oral Surg., Oral Med., Oral Pathol. **52**, 648, 1981.

15. Wierzbicka M.: Periodontologia kliniczna. Sanmedia-Med. Tour Press Intern., Warszawa 1992.

Otrzymano 1995.05.19.

EXPLANATIONS TO FIGURES

Fig. 1. Experimental group I. Light microscopic appearance odontoblastic layer. Hematoxylin and eosin staining. Magn. 300×.

Fig. 2. Experimental group I. Electron micrograph illustrating the odontoblasts with ovoid nucleus and chromatin granules. Intracellular organelles presents in cytoplasm. N — nucleus, Ch — chromatin, C — cytoplasm, M — mitochondria. Magn. 7000×.

Fig. 3 Experimental group I. Electron micrograph of odontoblast and myelinated axon and collagen fibers. O — odontoblast, N — nucleus, CF — collagen fibers, mA — myelinated axon. Magn. 7000×.

Fig. 4. Experimental group I. Electron micrograph of dental pulp. It consists of the fibroblasts dispersed in an extracellular compartment. N — nucleus, mA — myelinated axon. Magn. 7000 ×.

Fig. 5. Experimental group I. Scanning electron micrograph of the odontoblasts. Notice the collagen fibers. Magn. 2000×.

Fig. 6. Experimental group II. Light microscopic appearance of the odontoblastic layer. Hematoxilin and eosin staining. Magn. 300×.

Fig. 7. Experimental group II. Electron micrograph of the odontoblasts shows vacuolation of cytoplasm and their nucleus show irregular nuclear membrane and severe chromatin granules. N — nucleus, Ch — chromatin, V — vacuole. Magn. 5000×.

Fig. 8. Experimental group II. Electron micrograph of the odontoblast with nucleus containing severe chromatin clumping. Next to it there are two myelinated axons. N — nucleus, Ch — chromatin, mA — myelinated axon. Magn. 5000×.

Fig. 9. Experimental group II. Electron micrograph of dental pulp from fibroblast and macrophages. Ma — macrophage, N — nucleus. Magn. 5000×.

Fig.10. Experimental group II. Scanning electron micrograph of odontoblasts. Magn. 2000×.

STRESZCZENIE

Przeprowadzono badania ultrastrukturalne nabłonka odontoblastycznego miazgi zębów w zależności od wieku pacjenta. Wyniki badań wskazują, że zmiany morfologiczne występujące w odontoblastach związane są z procesami starzenia się komórek. Dotyczą one zmniejszenia liczby komórek w nabłonku odontoblastycznym, wakuolizacji komórek, zmian strukturalnych mitochondriów i chromatyny jądrowej.

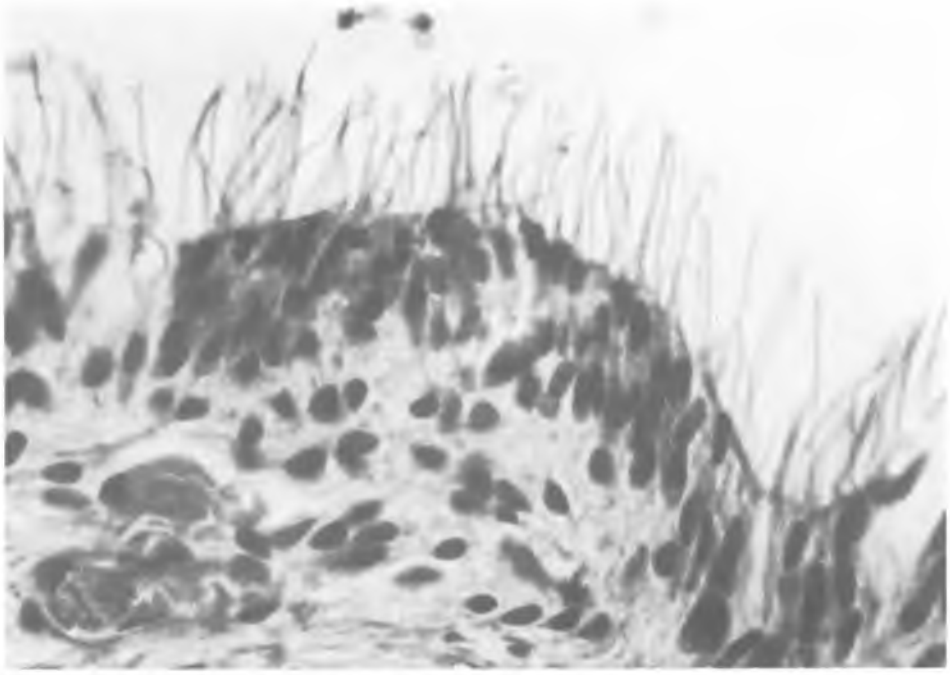

Fig. 1

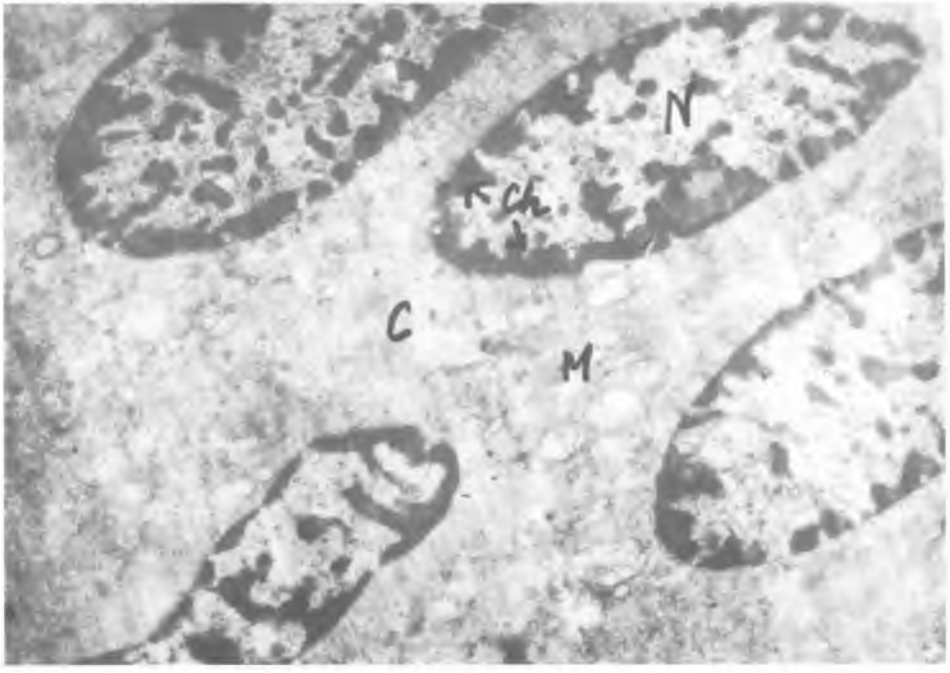

Fig. 2

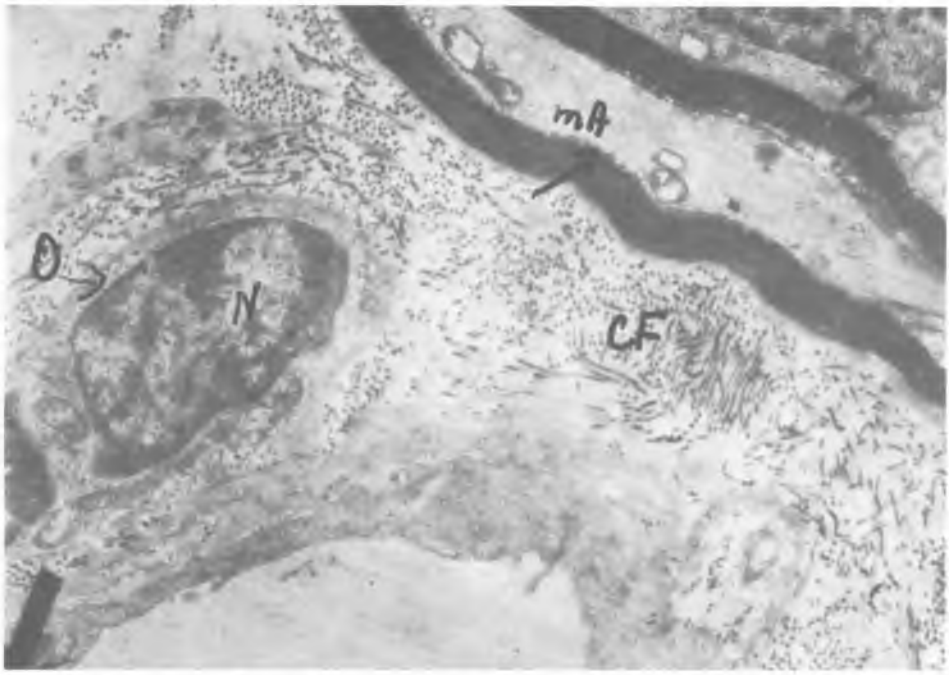

Fig. 3

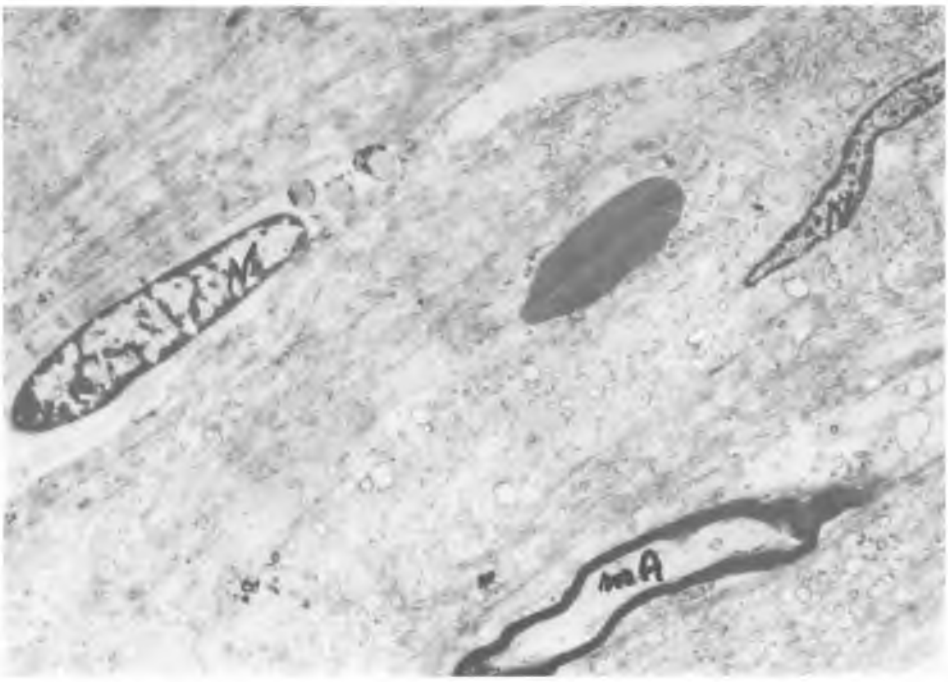

Fig. 4

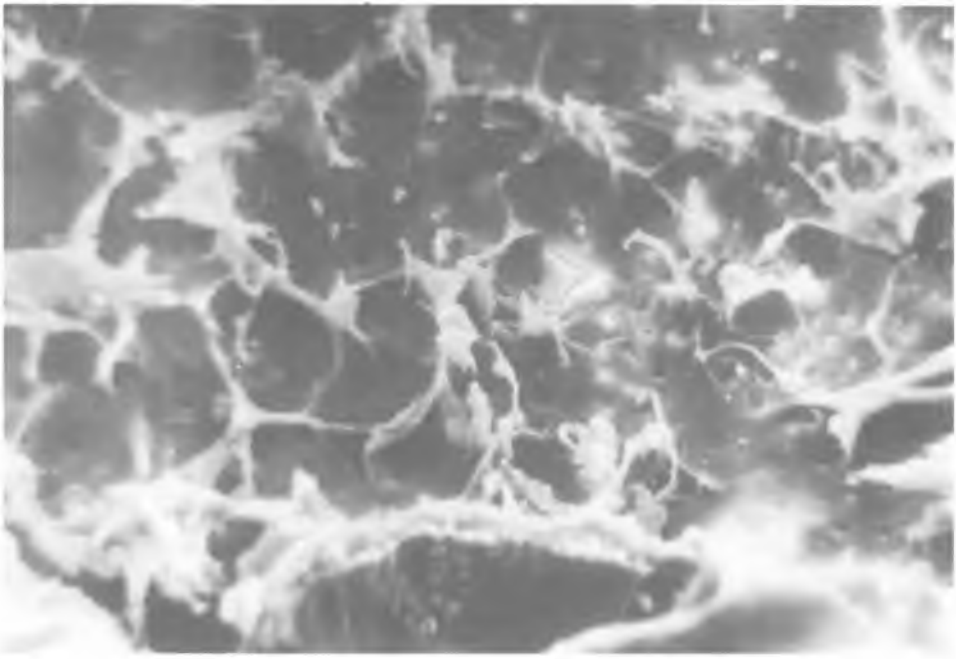

Fig. 5

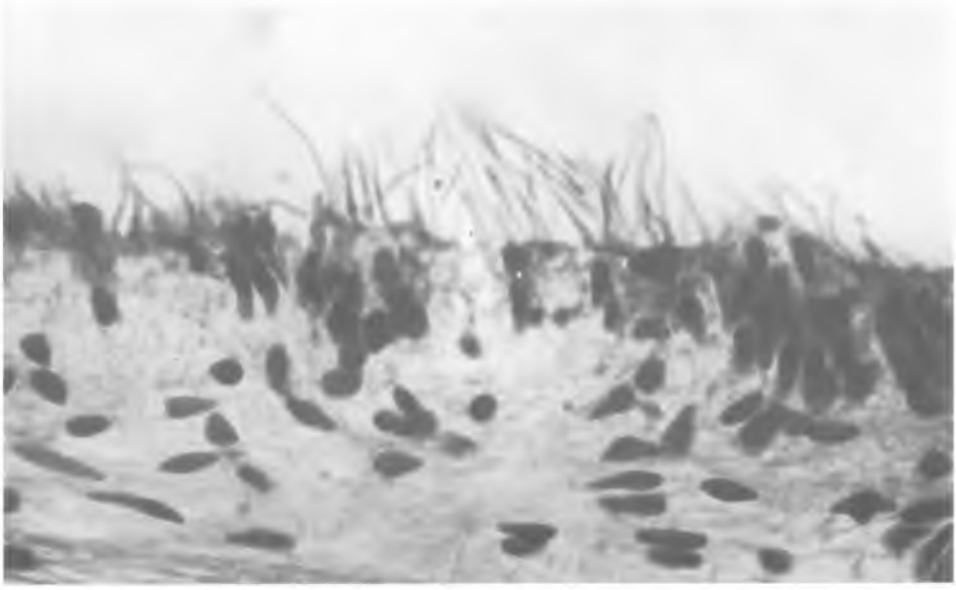

Fig. 6

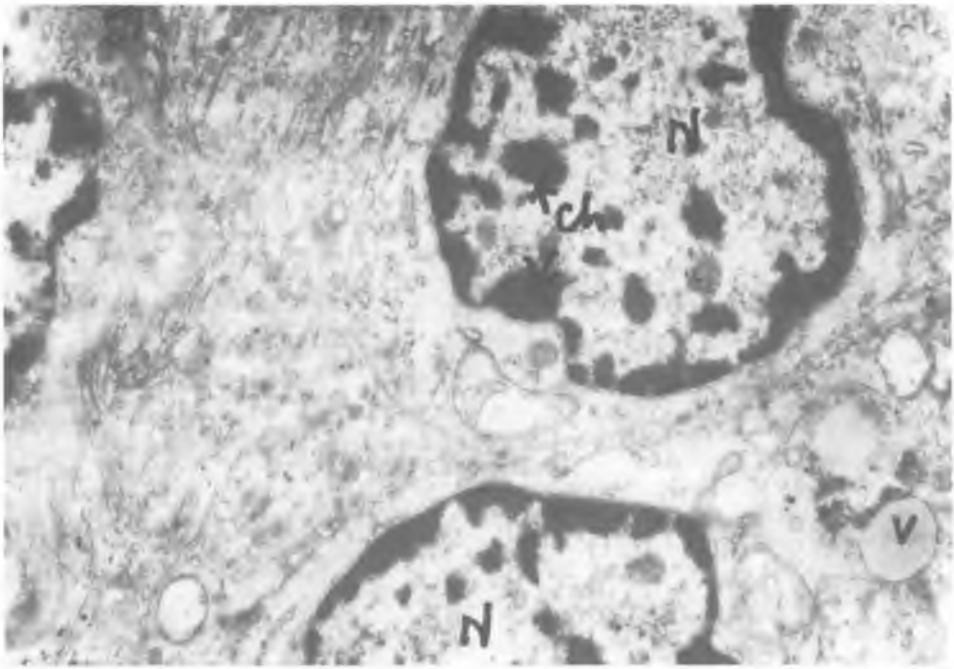

Fig. 7

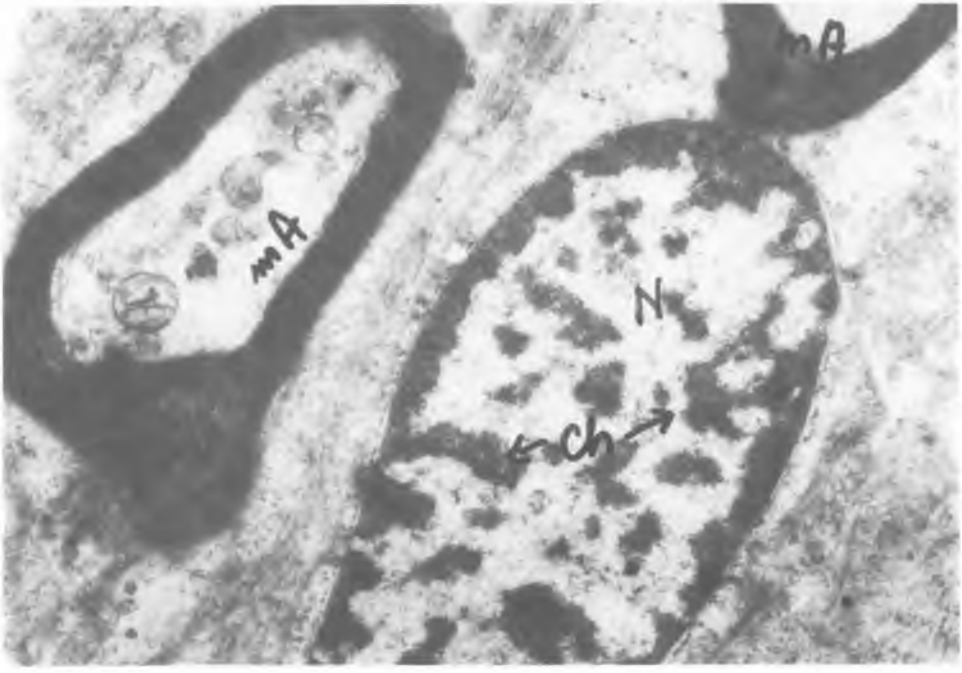

Fig. 8

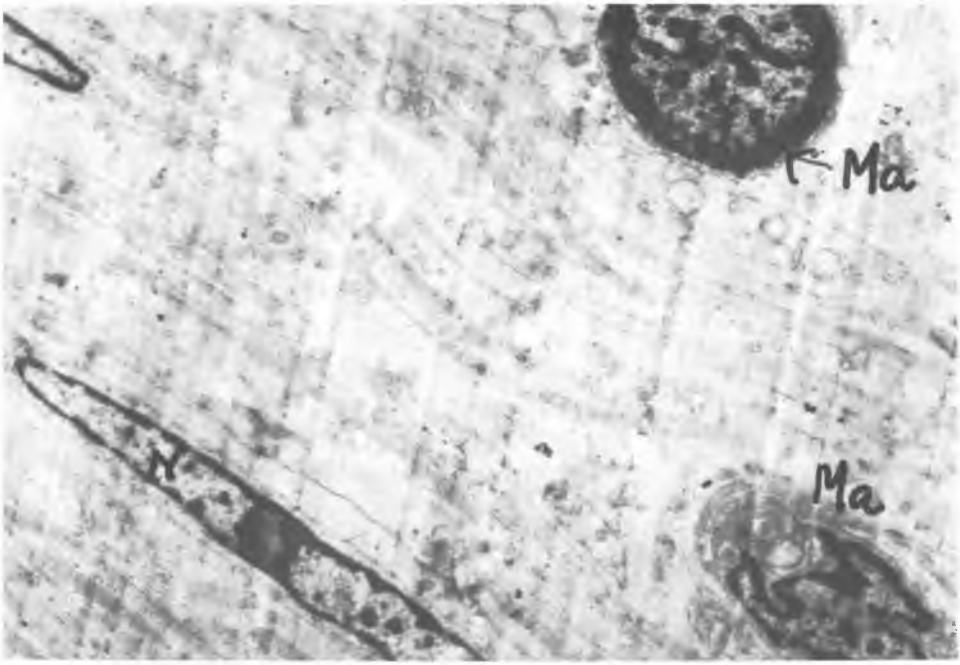

Fig. 9

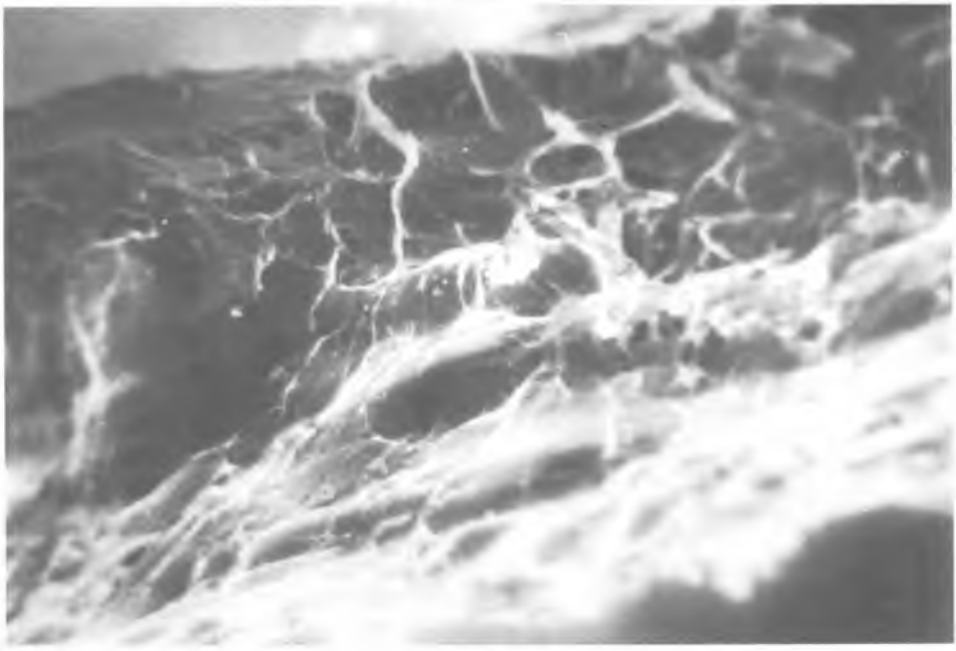

Fig. 10