ANNALES
UNIVERSITATIS MARIAE CURIE-SKŁODOWSKA
LUBLIN — POLONIA
VOL. XLII, 11 SECTIO D 1987

Katedra i Zakład Histologii i Embriologii z Pracownią Cytologii Doświadczalnej. Akademia Medyczna w Lublinie
Kierownik: prof. dr hab. n. med. Irena Królikowska-Prasał

Jadwiga ROMANOWSKA-SARLEJ

Ultrastructure of Embryonic Germ Cells of the Chicken Experimentally Treated with Estradiol Benzoate

Ultrastruktura komórek płciowych kurcząt w warunkach doświadczalnego podania benzoesanu estradiolu w okresie embrionalnym

Ультраструктура половых клеток цыплят в опытных условиях применения бензоата эстрадиола в эмбриональный период

Many authors have reported their findings on the influence of female sex hormones on the development of embryonic bird gonads (4, 8, 9, 12). These steroids have the greatest effect on the differentiation of the male gonads. The reports also emphasize the role of estrogenic hormones in gonad histogenesis (5).

The purpose of the present experiment was to investigate the effect of estradiol benzoate administered during gonad differentiation upon the ultrastructure of germ cells.

MATERIALS AND METHODS

60 chicken embryos (obtained from the Lublin Chicken Hatchery) were used, which received into the yolk 0.15 μg of estradiol benzoate in an oil solution on the 7th day of incubation. The experiment was performed in 3 groups: control group I received nothing, control group II received 0.03 ml of oil solution. Group III was experimental — it receoived the hormone. After hatching the cortex of the left female gonads and both male gonads were taken for examination. The ultrastructure of germ cells was examined using the electron microscope according to the following technique: sections were fixed for 2 hrs in 6.25% glutaraldehyde in 0.2 M cacodyl buffer at *pH* 7.2. The sections were then washed in a cacodyl buffer at the same *pH* and the material was postfixed in 1% osmium tetroxide in 0.2 M cacodyl buffer at *pH* 7.2. The sections were then dehydrated and embedded in Epon 812. Thick sections were polymerized at 60°C and cut on a Tesla BS 490 ultramicrotome. Ultra-thin sections were stained with uranyl acetate and with lead citrate. They were studied and photographed with a Tesla BS 613 electron microscope.

RESULTS

THE OVARY

The cortex of the ovaries of the control animals (groups I and II) showed a similar structure. It contained oogonia of about the same size and larger oocytes with intercellular bridges, and accompanying prefollicular cells.

The oocyte nucleus, usually rounded, was large in proportion to the cell size. It contained nucleopores, stroma of evenly distributed heterochromatin and nucleolus (Fig. 1). The cytoplasm of the oocytes contained a vitelline nucleus lying close to the nucleus, free mitochondria, ribosomes and polyribosomes. Granular membranes, small follicles of smooth membranes, microfilaments or lipid vacuoles were a rare occurrence. Oocytes in the meiotic prophase (Fig. 2) and degenerative forms of oocytes were observed.

At the electron microscope level prefollicular cells differed from germ cells by denser hyaloplasm. They contained a large nucleus, mitochondria, weak RER membranes and many follicles (Figs. 1 and 2).

After estradiol administration in group III more numerous germ cells in the division stage were observed in the cortex of the experimental ovaries. The submicroscopic picture of the oocytes showed the widening of the cisternae of endoplasmic reticulum (Fig. 3) and of the perinuclear space at some places (Fig. 4). In the cytoplasm of oocytes there were more numerous occurrences of lipid vacuoles and sporadic of swollen mitochondria. In the prefollicular cells an increase in the amount of RER and ribosomes could be observed.

THE TESTIS

The seminiferous tubules in the control groups had no lumen. The cell population of the tubules was made up of spermatogonia and more numerous Sertoli cells. Spermatogonia lay singly on the circumference of the tubulae or closer to its centre (Fig. 5). They contained the eccentrically located nucleus with a nucleolus. In the abundant cytoplasm there were mitochondria, many of them in the neighbourhood of the nucleus and the Golgi apparatus was formed of fine cisternae and follicles. The cytoplasm of spormatogonia also contained polyribosomes, short ER cisternae, small follicles, microfilaments, multivesicles bodies and very few lipid vacuoles.

Sertoli cells, irregular in shape, lay on the base membrane of the tubulae. They were characterized by the nucleus in folds with deep concavities. The nucleolus, one to three in number, was formed of granular elements. The cell cytoplasm contained mitochondria with a dark matrix, ER cisternae, the Golgi apparatus, ribosomes and polyribosomes, dense bodies, clusters of thin microfilaments, and lipid vacuoles. Two types of Sertoli cells were observed: with dark and light cytoplasm at the electron microscope level.

After estradiol injection on the 7th day of incubation the morphological metamorphosis of the left gonads occurred. The peripheral, ovary-type cortex appeared in the feminized organ. The remainder of the gonad had a tubular structure. The submicroscopic picture of the newly formed cortex was similar to that of the cortex of the ovary of control animals, the difference being that most germ cells were in the interphase stage (Fig. 6).

In the spermatogonia more abundant RER membranes with short and widened cisternae were observed (Fig. 7). There were very few swollen mitochondria and myelin structures in the cytoplasm. Degenerative forms of spermatogonia were also found.

In Sertoli cells the RER cisternae were widened and there were more frequent occurrences of dense bodies and lipid vacuoles.

DISCUSSION

The present observations on the morphology and ultrastructure of the examined bird gonads confirm the results of other experiments (1, 3, 6, 7, 10, 11).

In the present experiment, after exogenic estrogens were administered, more numerous germ cells in the division stage were reported in the animals ovaries. In the ultrastructure of the oocytes the following changes were observed: the widening of the ER cisternae and the perinuclear space and a greater amount of lipid vacuoles. An increased number of follicles and SER cisternae and their metamorphosis into lipid drops has been observed by Bielańska-Osuchowska (2) in the maturing oocytes in the swine. More numerous occurrences of lipid vacuoles in the present experiment may also be due to estradiol administration in an oil solution.

In the prefollicular cells an increase in the number of RER was reported. Since the bird oocytes are large cells, surrounded by numerous follicle cells, it is possible that the latter play a role in the preparation of oocytes for yolk production. The increased number of RER and ribosomes in the prefollicular cells, developing into follicle cells, was also observed by Narbaitz (10) in 4-day chickens after estradiol treatment.

In the ovotestis of the experimental group there was a cortex similar to that of the female control gonad. The only difference was that most germ cells were in the interphase stage. The rate of propagation of genetically male germ cells also turned out to be higher than that of male control embryos. In the submicroscopic picture of spermatogonia and Sertoli cells an increased number of RER membranes and lipid vacuoles was observed. Since there were degenerative spermatogonia in the tubules, it is possible that they accounted for the occurrence of an increased amount of lipids in Sertoli cells, although the effect of estradiol itself should also be taken into consideration.

These results permit to infer that estradiol administration together with the reversal of the left male gonads into the ovotestis accelerated the division of germ cells in the gonads. The increased amounts of ribosomes and RER elements in the examined cells may indicate stimulated protein synthesis there.

REFERENCES

1. Bellairs R.: The Relationship between Oocytes and Follicle in the Hen's Ovary as Shown by Electron Microscopy. J. Embryol. Exp. Morph. **13,** 215, 1965.
2. Bielańska-Osuchowska Z.: Ultrastruktura pierwotnych pęcherzyków jajnikowych u płodów świni (*Sus scrofa* d.), XII Zjazd PTA, Kraków 1980.
3. Carlon N., Erickson G. F.: Fine Structure of Prefollicular and Developing Germ Cells in the Male and Female Left Embryonic Chick Gonads *in vitro* with and without Androgenic Steroids. Ann. Biol. Anim. Bioch. Biophys. **18,** 335, 1978.
4. Dantchakoff V.: Sur l'inversion sexuelle expérimentale de l'ébauche testiculaire chez l'embryon du poulet, C. S. Acad. Sci. Paris 1983–1985, 1935, 200.
5. Gasc J. M.: Estrogen Target Cells in Gonads of Chicken Embryo during Sexual Differentiation. J. Embryol. Exp. Morph. **55,** 331, 1980.
6. Gilbert A. B.: The Ovary in Physiology and Biochemistry of the Domestic Fowl, Ed. by D. J. Bell and B. M. Freeman, **3,** London–New-York 1971.
7. Greenfield M. L.: The Oocytes of the Domestic Chicken Shortly After Hatching, Studied by Electron Microscopy. J. Embryol. Exp. Morph. **15,** 297, 1966.
8. Jordanov J.: Entrée en préméiose des cellules germinales du testicule embryonnaire du poulet. C. R. Acad. Bulg. Sci. **27,** 1443, 1974.
9. Łukina A.: Opriedielenije pierioda czuwstwitielnosti połowych kletok kurinogo embryona k' diejstwiju estradiol-bienzoata. Cytołogia **20,** 903, 1978.
10. Narbaitz R.: Ultrastructural Aspect of Cortical Differentiation in Chick Ovaries and Intersexul Gonads. Z. Zellforsch. Microsc. Anat. **118,** 315, 1971.
11. Samer M. E., Avila R. E., de Fabros S. P.: Cytochemical Study of the Ovary Cells in the Chick Embryo. Folia Histoch. et Cytoch. **21,** 3–4, 173, 1983.
12. Wolff E.: Le rôle des hormones embryonnaires dans la différenciation sexuelle des oiseaux. Arch. Anat. micrósc. Morph. exp. **39,** 426, 1950.

EXPLANATION TO FIGURES

Fig. 1. Control group. Ovary. Oocyte (Oo), prefollicular cell (Fc), cell membrane (Cm), mitochondria (M), nucleus (N), nucleolus (Nu), rybosomes (R), rough endoplasmic reticulum (RER), nucleopores (arrow). Magn. 14,000 ×.

Fig. 2. Control group II. Ovary. Oocyte (Oo) in a division and prefollicular cell (Fc). Cell membrane (Cm), nucleus (N), filaments of chromosomes (Ch), rybosomes (R), vacuoles (V), nucleopores (arrow). Magn. 14,000 ×.

Fig. 3. Experimental group. Ovary. Oocyte (Oo). Mitochondria (M), nucleus (N), endoplasmic reticulum (ER), filaments (Fi). Magn. 30,000 ×.

Fig. 4. Experimental group. Ovary. Oocyte (Oo). Nucleus (N), mitochondria (M), perinuclear space (arrows). Magn. 35,000 ×.

Fig. 5. Control group I. Testis. Spermatogonium (Sg), Sertoli cell (Scl), mitochondria (M), nucleus (N), nucleolus (Nu), rough endoplasmic reticulum (RER), multivesicular bodies (Mvb), Golgi apparatus (G). Magn. 14,000 ×.

Fig. 6. Experimental group. Cortex of left tesits. Oocyte (Oo), prefollicular cell (Fe). Golgi apparatus (G), membrana cell (Cm), nucleus (N), mitochondria (M), multivesicular bodies (Mvb), rybosomes (R), vesicles (Ve). Magn. 14,000 ×.

Fig. 7. Experimental group. Testis. Spermatogonium (Sg), Sertoli cell (Scl). Mitochondria (M), nucleus (N), nucleolus (Nu), rough endoplasmic reticulum (RER). Magn. 14,000 ×.

STRESZCZENIE

Zarodkom kur podano dożółtkowo 0,15 μg benzoesanu estradiolu w siódmym dniu inkubacji. Po wylęgu pobrano korę lewych gonad żeńskich oraz obie gonady męskie do badań w mikroskopie elektronowym. Stwierdzono, że podany estradiol wywołał zmiany w obrazie submikroskopowym komórek płciowych. W oocytach zmiany dotyczyły poszerzenia kanałów cystern RER i przestrzeni okołojądrowej. Zauważono liczniejsze komórki płciowe w stadium podziału. W spermatogoniach nastąpił wzrost ilości cystern RER, które przybrały wygląd krótkich i poszerzonych kanałów.

РЕЗЮМЕ

Куриный эмбрион на 7-ой день инкубации получал в желток 0,15 μg бензоата эстрадиола. После вылупления взято кору левых женских гонад и две мужские гонады для исследования в электронном микроскопе. Определено выступление изменений в субмикроскопической картине половых клеток вызванных эстрадиолом. В ооцитах выступило расширение каналов цистерн RER и околоядерного пространства. Замечено также увеличение половых клеток в стадии деления. В сперматогониях выступил рост количества цистерн RER, которые выглядели как короткие расширенные каналы.

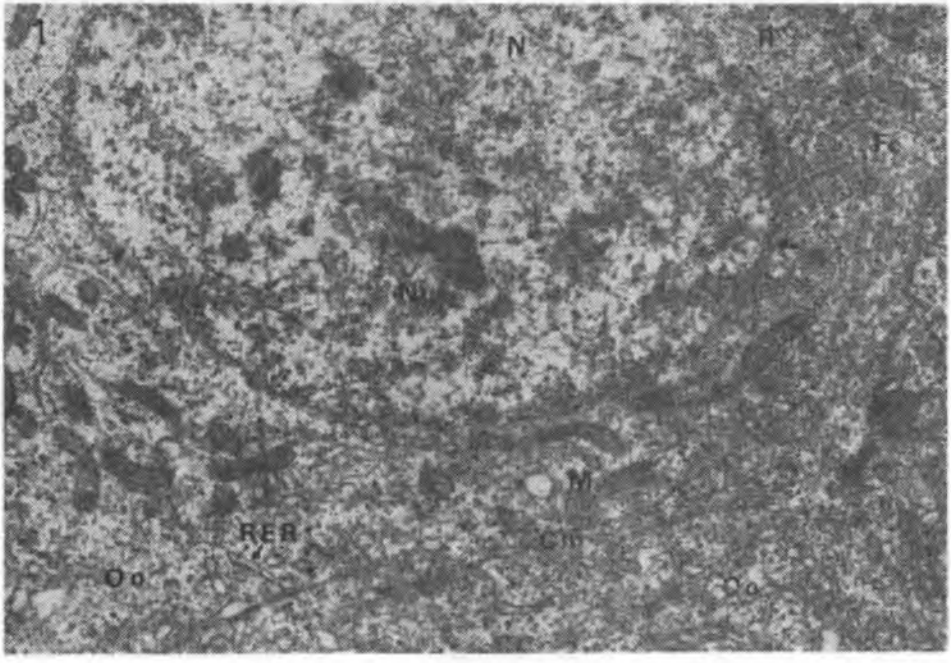

Fig. 1

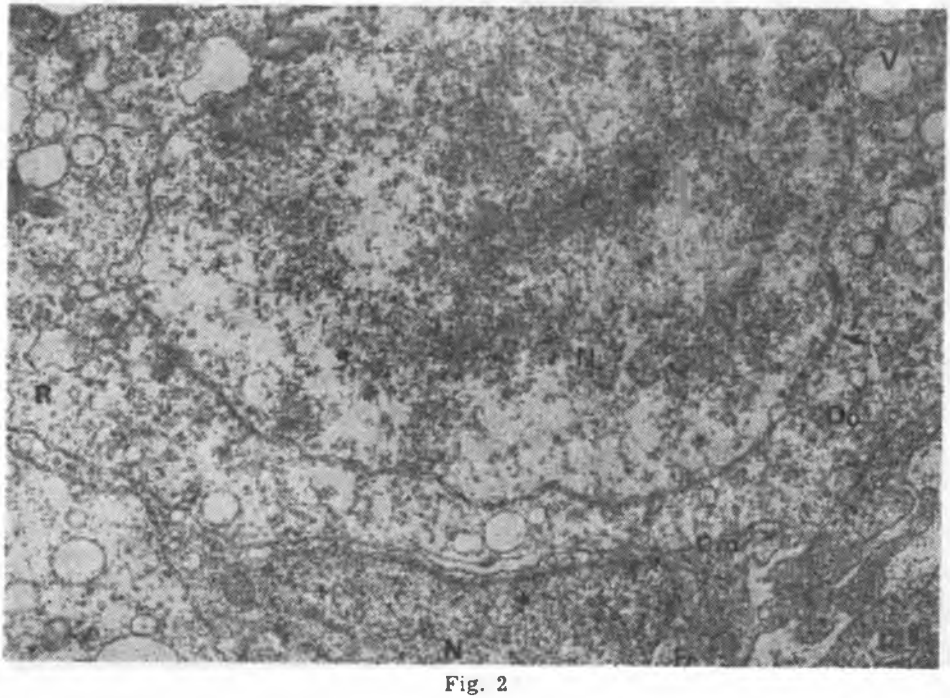

Fig. 2

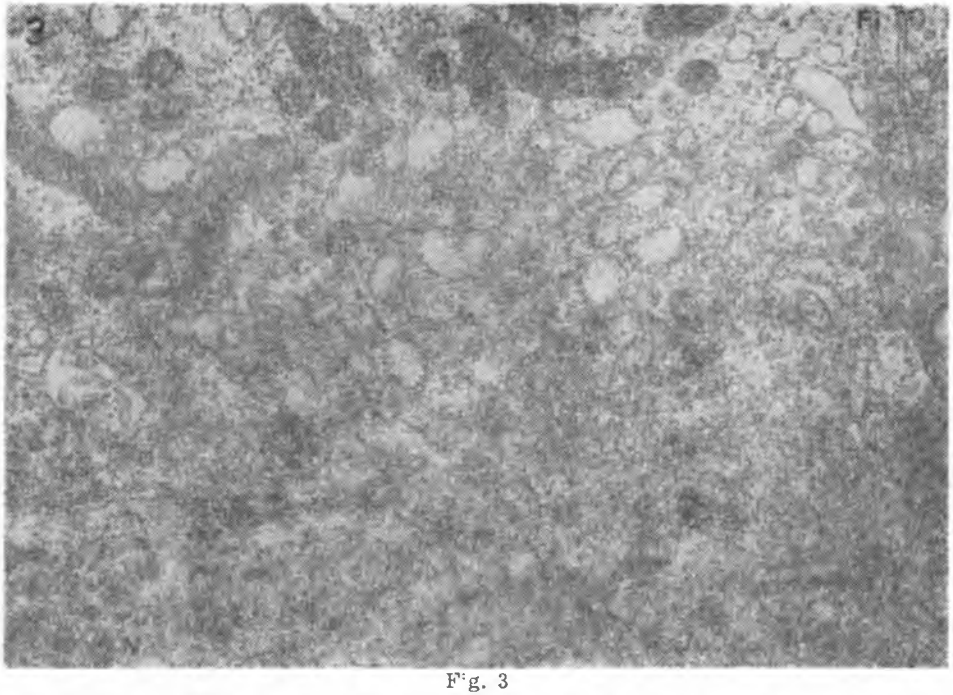

Fig. 3

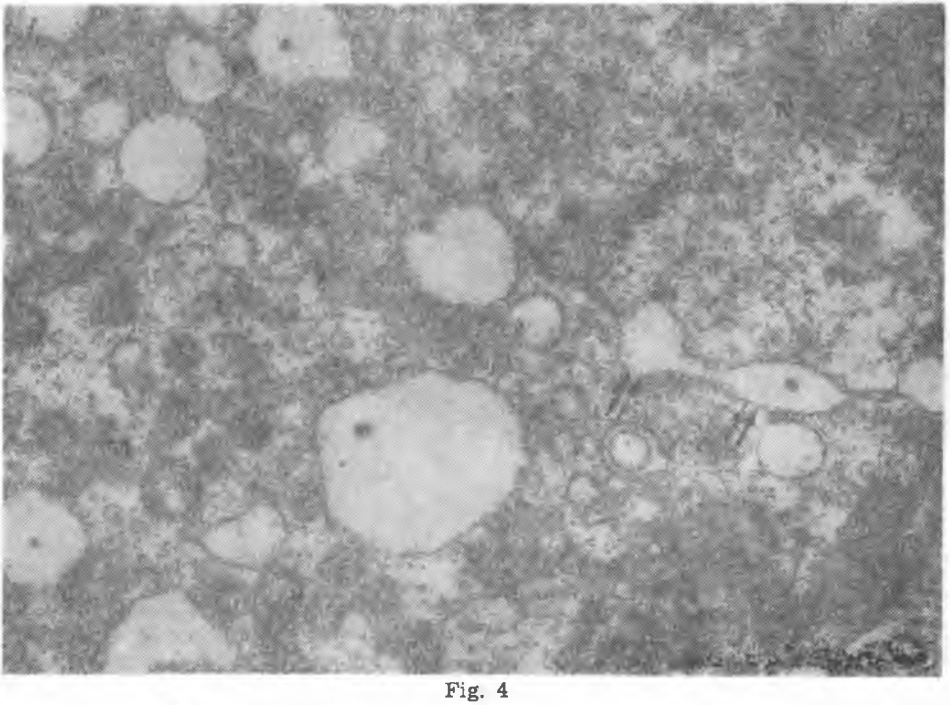

Fig. 4

Jadwiga Romanowska-Sarlej

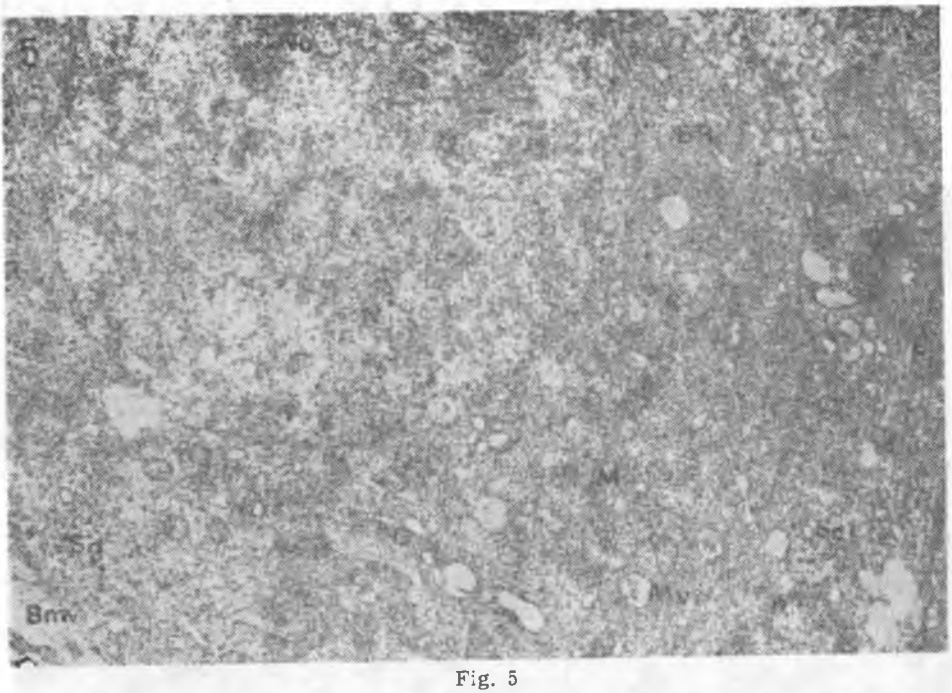

Fig. 5

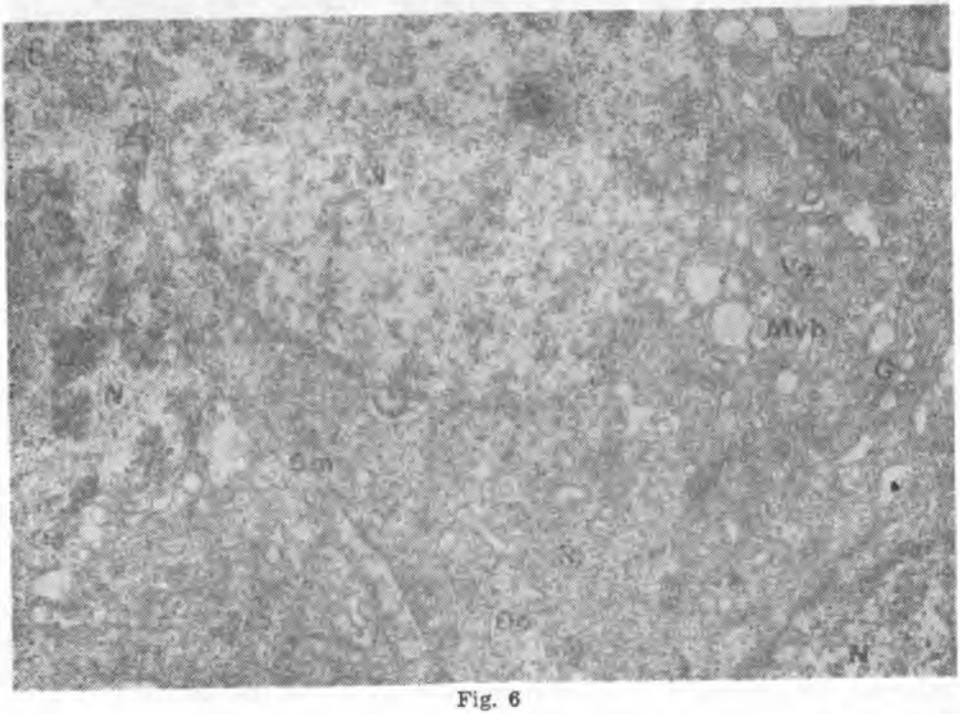

Fig. 6

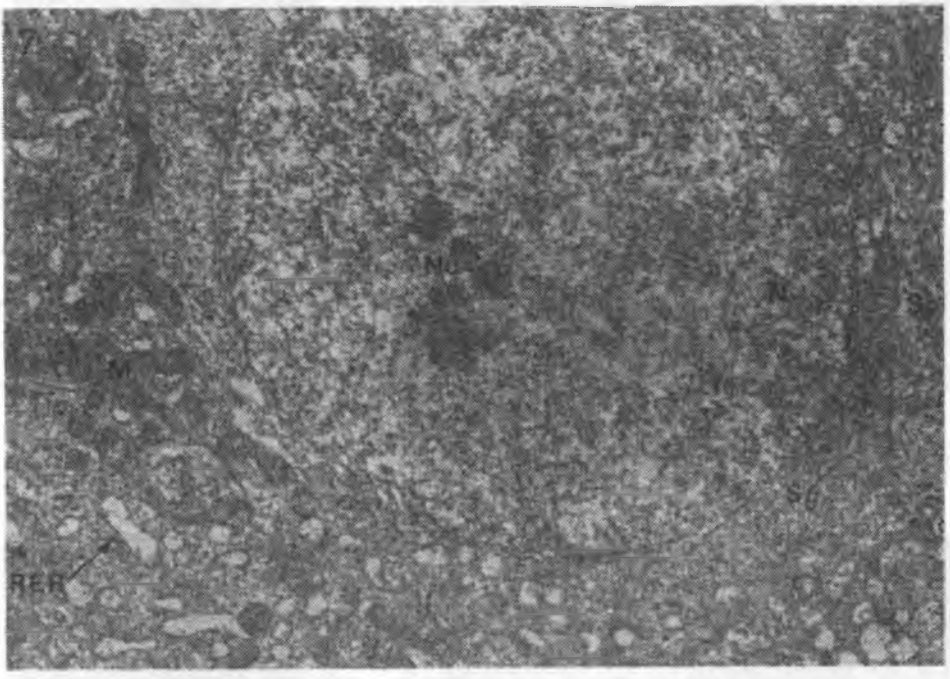

Fig. 7