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Ultrastructural Studies on Mitochondria of Preimplantaion Rabbit Embryos

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ABSTRACT

The ultrastructural changes of mitochondria in the ovarian oocytes from Graafian follicles, the ovulated tubal ova, and the various stages of preimplantation rabbit embryos have been observed with an electron microscope.

From the ovarian oocytes to the 4-cell stage, mitochondria showed oval and round forms with a few cristae arranged concentrically and peripherally at the inner membrane. In 8-cell and 16-cell stages, mitochondria tended to change their forms to be elongated, and their sizes, and the outer membrane of the mitochondria had a tendency to become rough and irregular although there were few changes in the inner structure. In morula, some mitochondria began to show several transverse cristae proceeding into the matrix. Mitochondria rapidly increased in number at the late blastocyst stage. Matrix of mitochondria with transverse cristae found in the morula and in blastocyst stages was less dense than that of the earlier stages. The authors believe that the morphological changes of mitochondria during early embryonal development indicate the level of enzymatic activity at which this organelle is engaged in energy metabolism.

INTRODUCTION

The successful culture of rabbit ova from zygote to blastocyst has been accomplished by a number of investigators (Suzuki, 1966; Mauer et al., 1968; Kane and Foote, 1970; Brinster, 1970). The fact that the energy metabolites required by the preimplantaion rabbit embryos undergo several changes during development *in vitro* is also evident from the results of investigations by Daniel(1967). Fridhandler(1968), and Brinster(1970), It is verified that the metabolism pattern also changes (Daniel, 1967; Fridhandler, 1961; Fridhandler et al., 1957), and that these changes in metabolism involve structural changes in the mitochondria during oogenesis and cleavage of the early embryos

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(Stern et al., 1971).

Recently, many authors have reported on the ultrastructure of mammalian oocytes and early embryos (Blanchette, 1961; Schlafke and Enders, 1963; Zamboni and Mastroianni, 1966; Calarco and Brown, 1968; Hillman and Tasca, 1969; Stern et al., 1971). However, few papers have deeply discussed the correlationship between the morphological changes of mitochondria and the energy metabolic alteration during cleavage of the rabbit embryos.

The purpose of the present experiment is to observe the structural changes of mitochondria of the oocyte and preimplantation stage of the rabbit embryos, in order to verify the correlation between the changes and energy metabolism.

MATERIALS AND METHODS

Domestic rabbits weighing 1.2-2.0 kg were used in this experiment. The ovarian oocytes were collected from Graafian follicles in the ovaries by the method of Donahue(1968). For the collection of the ovulated tubal ova, the does were superovulated by a subcutaneous injection of 50 i.u. of PMS (pregnant mare's serum gonadotrophin, Gestyl, Organon) per day for three days, followed, 24 hours later, by an intravenous injection of 100 i.u. of HCG (human chorionic gonadotrophin, Equinex, Ayerst). The tubal ova were harvested by flushing the fallopian tubes of the superovulated does with the culture medium (TC medium 199) 14 hours after final hormone injection. The same method as described above was employed for the collection of the fertilized ova and the various stages of preimplantation embryos, except the does were mated by a fertile buck just before injection of HCG. The embryos were harvested by flushing the genital tracts at the appropriate time.

For electron microscopy, the oocytes and embryos were immediately prefixed for 30 min. in a 3% glutaraldehyde solution buffered (pH 7.4) with 0.1M phosphate buffer, and washed two times for 20 minutes each with phosphate buffer. The materials were then postfixed for 30 min. in a 1% osmium tetroxide buffered (pH 7.4) with 0.1M phosphate buffer. After double fixation the materials were rapidly dehydrated in ethanol series and prophylen oxide, and embedded in Epon 812 (Luft. 1961). The thin sections were cut with Sorvall MT2 Porter-Blum ultramicrotome, and stained with saturated uranyl acetate (Watson, 1961) followed by lead citrate (Venable and Coggeshall, 1965), and examined under a Hitachi HU-11E-1 electron microscope operated at an accelerating voltage of 75KV.

OBSERVATIONS

In several sections of the rabbit embryos the plasma membrane contact between two blastomeres was relatively smooth. But, there were many ooplasmic processes protruding towards the space between blastomeres (Fig. 4). The organelles such as the nucleus, Golgi complex, endoplasmic reticulum and mitochondria, and inclusions such as the lipid droplets and ribosomes, were generally found in ooplasm. Mitochondria were randomly distributed in the ooplasm of blastomeres through out all embryonic stages. Mitochondria in the ovarian oocytes obtained from Graafian follicles, the ovulated unfertilized ova, the fertilized ova, and the 2- or 4-cell embryos, showed round or oval forms with a relatively unique size. The mitochondrial cristae were rarely developed and were

arranged circumferentially to the inner membrane (Fig. 1, 2, 3).

In 8-cell and 16-cell embryos, mitochondria showed some diversity in shape and size (Fig. 5). Some mitochondria were elongated, and the others rough and of various sizes. The surface of the mitochondria showed a tendency to become rough and irregular(Fig. 6). Some showed small intracristal spaces arranged peripherally. Cristae were developing slightly, but were circumferential and concentrical to the inner membrane at this time. There were little differences in the inner structure when compared with that of the earlier stages. At these stages many mitochondria were associated with endoplasmic reticulum (Fig. 6).

In morula and early blastocyst stages some of the mitochondria became more developed with cristae arranged transversely in the matrix than that of the earlier stages, and the matrix was less dense. Other mitochondria still showed the primitive morphology notedin the earlier stages. Most of the mitochondria located near the Golgi complex were associated with the endoplasmic reticulum (Fig. 7, 8).

In mid and late blastocyst stages the mitochondria showing more elonated forms, were increased in number, and their cristae became more distinct and were arranged transversely in the matrix (Fig. 9, 10).

DISCUSSION

It has been known that mitochondria provides energy in the cell. Ample evidence that mitochondria are functionally organized at the molecular level has been accumulated since the works of Cooper and Lehninger (1956) and Davlin and Lehninger (1956). It is very interesting to verify the correlation between morphological changes of mitochondria and energy metabolic patterns concerned with the requirements for energy source during embryogenesis.

Anderson et al. (1970) observed in rabbit embryos that mitochondria, from the oocyte to all stages up to morula, have a simple and unique morphology. In the present studies, we found that the ovarian oocytes, ovulated ova and embryos from the fertilized one-cell to the 4-cell stage showed mitochondria having a simple morphology, as the observation of Anderson et al. However, at the 8- or 16-cell stage morphological changes began to appear in the mitochondria. These changes are comparable to those seen at 4- or 8-cell stage of mouse embryos(Stern et al., 1971). It should be noticed that mitochondria at these stages were somewhat diverse in shape and size (Fig. 5) and that the outer membrane showed a tendency to become rough and irregular (Fig. 5, 6). In morula stage a portion of mitochondria showed several transverse cristae proceeding into the matrix (Fig. 7). As the morula developed toward the late blastocyst stage the number of mitochondria with transverse cristae increased gradually.

There are also some differences *in vitro* in metabolic patterns between mouse and rabbit preimplantation embryos. The former requires pyruvate and lactate to support cleavage up to 4- or 8-cell stage and beyond these stages, glucose is preferentially utilized by embryos (Brinster, 1965 a, b; Brinster and Thomson, 1966; Biggers et al., 1970). However, for rabbits, although carbohydrates are not essential, amino acids are necessary for continuing cleavage (Daniel and Krishman, 1967; Daniel and Olson. 1969; Mauer et al., 1968; Brinster, 1970). From these findings, it might be assumed that amino acids and endogenous nutrients could be utilized as en-

ergy sources for cleavage in rabbit embryos. Stern et al. (1970) stated that the morphological changes of mitochondria in mouse embryos are interrelated with the changes in carbohydrate requirements. Even though the type of carbohydrate requirement is different between mouse and rabbit embryos, the changes of mitochondria during cleavage showed a similar pattern. That is, morphological changes of mitochondria begins to appear at 4- or 8-cell stages in mouse and at 8- or 16-cell stages in rabbit.

Daniel (1967) found that the 8-cell and 16-cell stages of rabbit embryos utilized 6-phosphogluconate, which does not support the earlier embryos. In addition to this, investigators (Brinster, 1968, 1969; Fridhandler, 1961) have found in the rabbit embryo that the ratio of CO_2 production from C_1 and C_6 labelled glucose for the preblastocyst stage was about 7-fold more than that for the blastocyst stage. These findings strongly suggest that a pentose shunt may be functional in the 8- or 16-cell stages in rabbit embryos hence this metabolic change might induce mitochondrial change as well, because, at these stages, primary changes in mitochondria can be seen at the space between outer and inner layer where adenylate kinase and nucleotide diphosphokinase are found. However, as yet, there has not been any data available to explain directly the mitochondrial changes in connection with such a physiological event. During the development from morula to blastocyst, mitochondria develop in form, and increase in number. Mitochondrial cristae increase and are arranged transversely in the matrix. Remarkable changes in the mitochondria at the blastocyst stage are concomitant with physiological changes that oxygen consumption and carbon dioxide production are rapidly increased. Some investigators found that oxygen consumption and carbon dioxide production by the preimplantation rabbit embryo markedly increased from the morula up to the blastocyst stages (Fridhandler, 1961; Mills and Brinster, 1967; Brinster, 1968, 1969). Oxygen consumption and carbon dioxide production by cells are very significant factors in interpretation of mitochondrial function. Therefore, from these findings in the rabbit embryo, one may conclude the respiratory chain activity is initially activated as a primary developmental event at the morula stage and rapidly increased up to the late blastocyst stage. The fact that the increase in the amount of glucose uptaken by embryo can be a signal that mitochondria have already changed their morphology from an inactive state to an active one. More investigations will be required to clarify the correlation between mitochondrial change and energy metabolic change during early development in rabbit embryos.

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Explanation of Figures

Fig. 1, 2, 3 and 4. From ovarian oocyte, tubal ovum, zygote, and 4-cell stage, respectively. Mitochondria (Mi) show round or oval forms, and have smooth outer membrane. Cristae are arranged concentrically and circumferentially to the inner membrane. The irregular ooplasmic processes (OP) proceed into the interspaces between the blastomeres.

Fig. 5 and 6. From 8-cell and 16-cell stage, respectively. Mitochondria (Mi) show some modified forms and sizes: Elongated and various sized mitochondria, some with rough surfaces. Some mitochondria (Mi) are associated with endoplasmic reticulum (↑).

Fig. 7 and 8. From morula and early blastocyt, respectively. Mitochondria (Mi) show more diversity in shape and size than that of the earlier stage. Some mitochondria (Mi) contain sevrael transverse cristae in the matrix and are closely associated with endoplasmic reticulum (↑).

Fig. 9 and 10. From mid and late blastocyst, respectively. Mitochondria (Mi) show more elongated forms. cristae are transversely arranged in the matrix.

Abbreviation: Go, Golgi complex; IS, Interspace between the blastomeres, Li, Lipid droplet; No, Nucleolus; Nu, Nucleus.

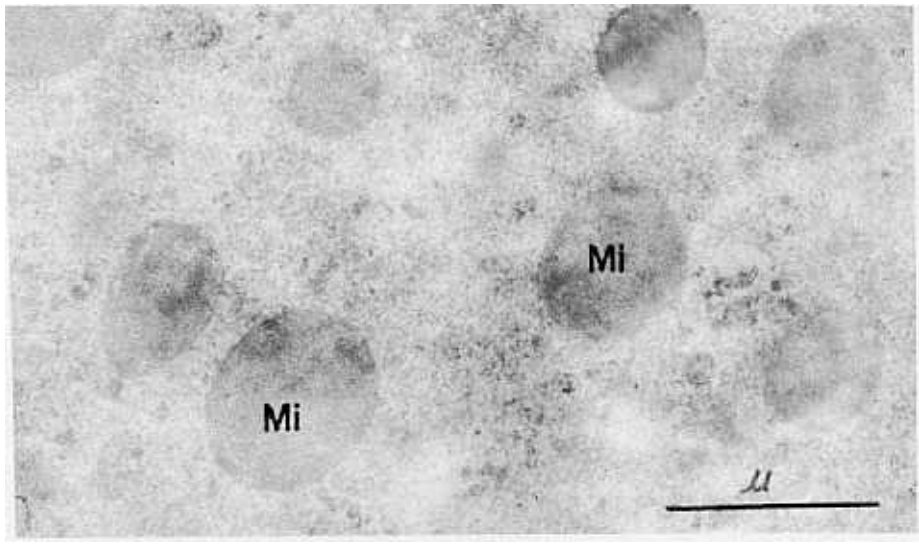
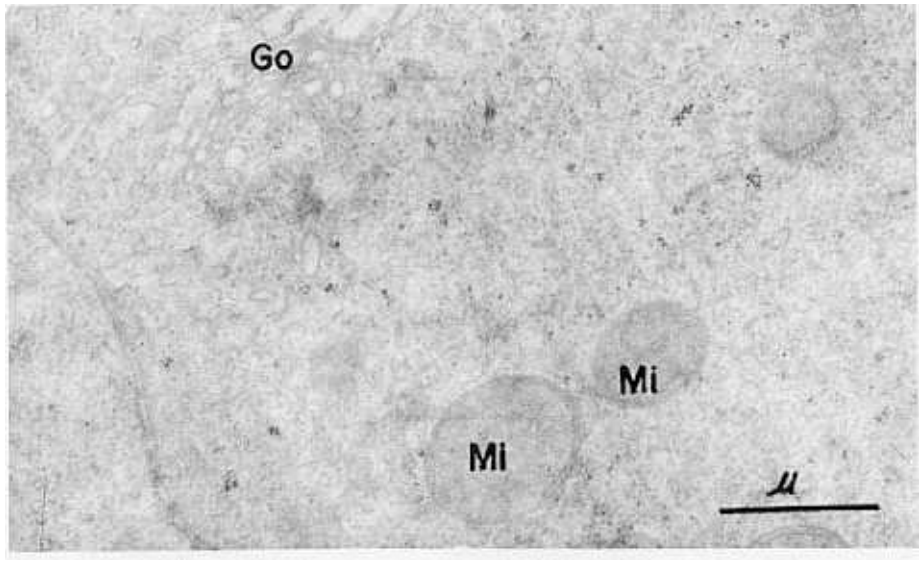


Fig.



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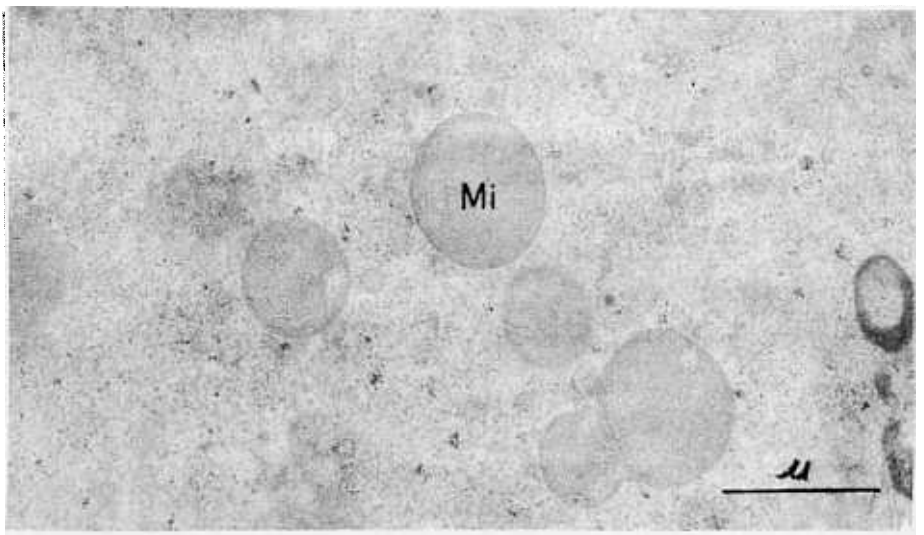

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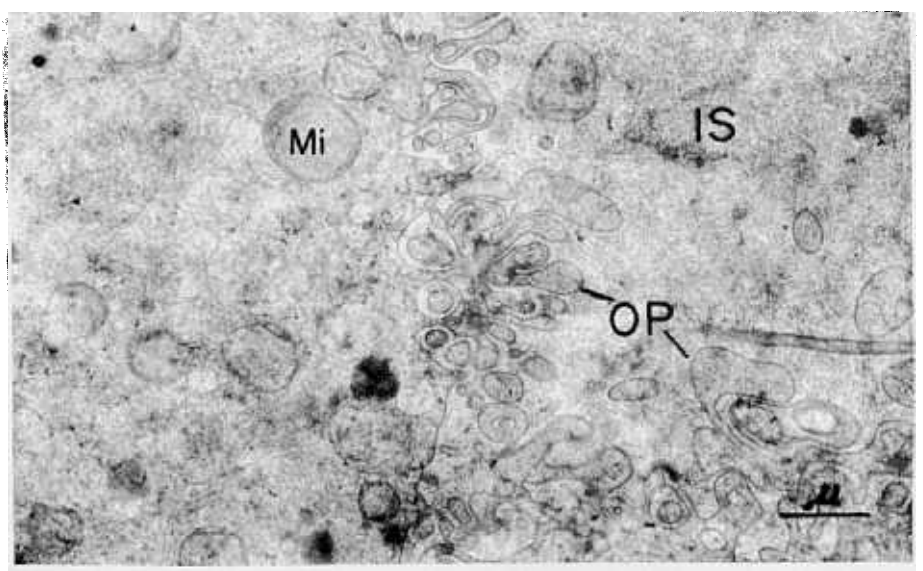


Fig. 4

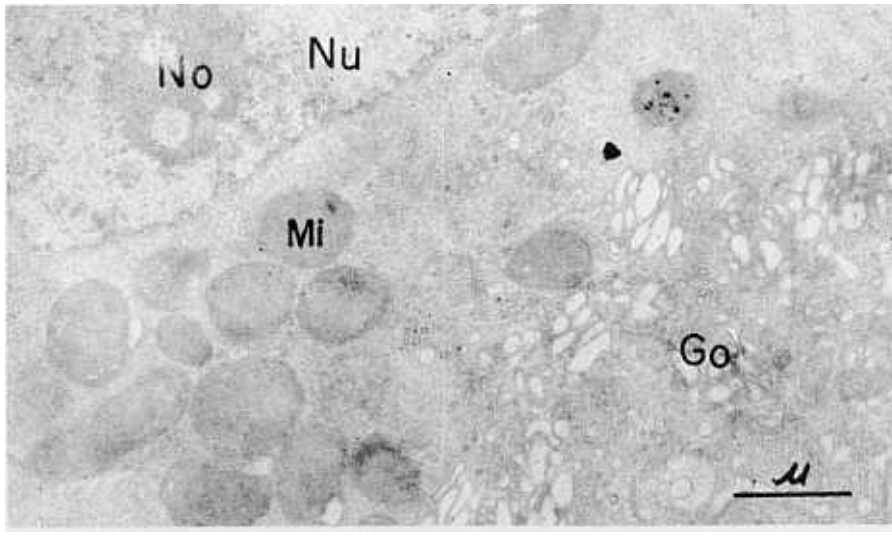
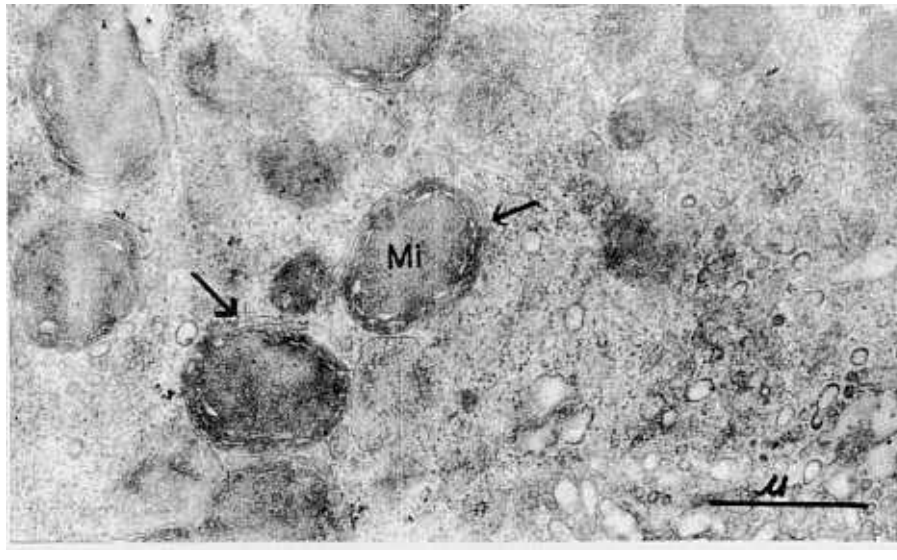


Fig. 5



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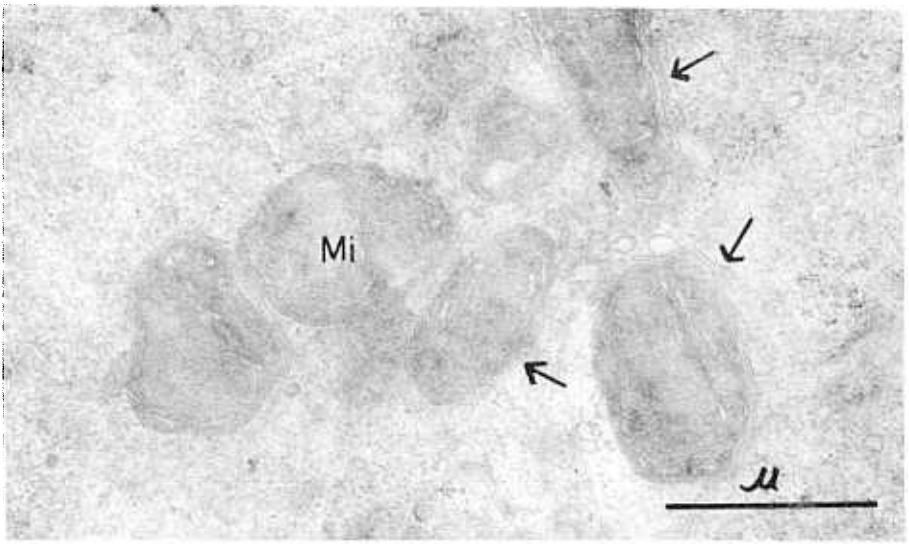

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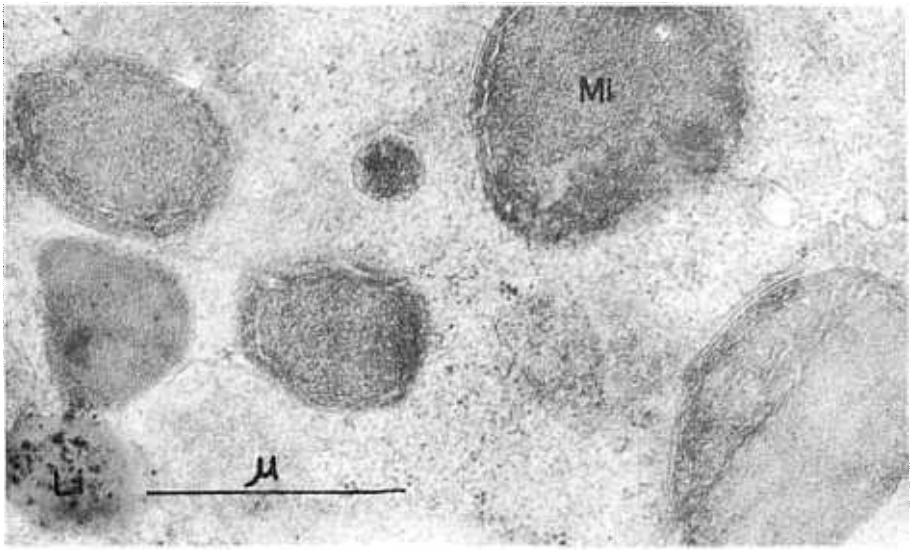

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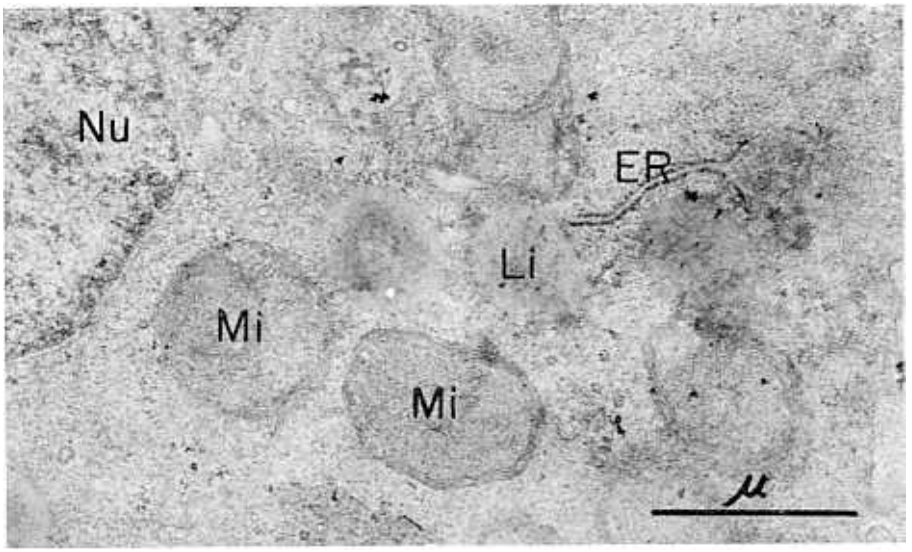

Fig. 9

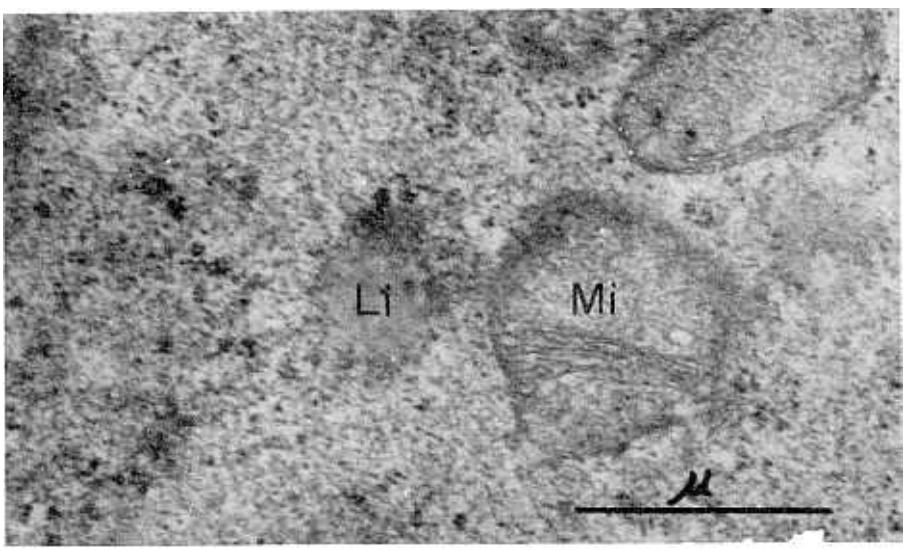

Fig. 10