THE ROLE OF MITOCHONDRIA IN THE OOCYTES OF MACACUS RHESUS AND CANIS FAMILIARIS

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In the oocytes of *Macacus rhesus* the mitochondria are granular in nature. The juxta-nuclear stage of mitochondria gives rise to perinuclear rings. Later a clear archoplasm is observed. When the egg grows in size and age, the layer of mitochondria breaks up into large number of patches, which arrange themselves in the form of a peripheral band. Later, these patches are observed scattered in the whole cytoplasm.

In *Canis familiaris* the mitochondrial aggregation is at first at the juxta-nuclear position; then they are seen concentrated at the yolk-nucleus area. Later, the mitochondria increase in number so much on one side that the nucleus is pushed away from the centre and becomes eccentric. The mitochondria, in the initial stages, are granular, but in advanced oocytes, they become finer and dust-like. Soon the cap of dust-like mitochondria disintegrates and the mitochondria are seen uniformly distributed in the ooplasm.

Introduction

Work carried out on mitochondria and their behaviour during gametogenesis so far shows that the mammalian group has received the least attention from modern cytologists. The present investigation was undertaken in order to study the behaviour of mitochondria in the oogenesis of monkey and dog.

Material and Method

For the demonstration of mitochondria, small pieces of ovary of freshly killed monkeys and dogs were fixed in Regaud-Tupa which yielded excellent results. In osmic fixatives all failed except the chrome osmium fluid of Champy. In Regaud-Tupa small pieces of ovary were kept for about 52 hours in cold, washed for half an hour in distilled water, then dehydrated as recommended in the Vade Mecum. The material was washed for twenty-four hours in running water, dehydrated and embedded.

The impregnation in the case of chrome-osmium fixative was carried out at 0°C. The solution was prepared as recommended in the Vade Mecum. The slides were stained with acid fuschsin, Aurantia and Toluidin blue. Iron-alum-haematoxylin did not give good results.

Experimental

Macacus rhesus.—The mitochondria in the case of monkey appear in the oogonium as extremely fine granules concentrated at the juxtanuclear position (Fig. 1). This mass at one pole of the nucleus next tends to become semiperinuclear and finally perinuclear in the form of a ring (Fig. 2). This perinuclear ring in the advanced oocyte breaks up into three prominent patches, of which

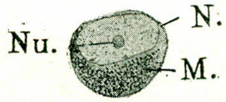

Fig. 1.

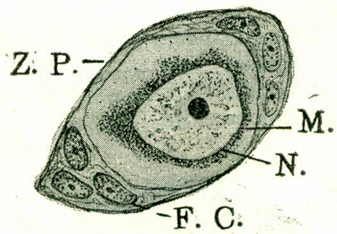

Fig. 2.

one is circular and appears homologous with the yolk nucleus of Balbiani, while the other two lie on the sides of the nucleus.

The mitochondria in the central circular patches leave that area, thus creating a clear rounded zone which may be called "archoplasm". Sometimes a centrosome is present in the archoplasmic area. In the next stage a medullary band of mitochondira round about the nucleus is seen. The extreme periphery is, at this stage, free from all inclusions (Fig. 3). As this egg grows in size and age, this

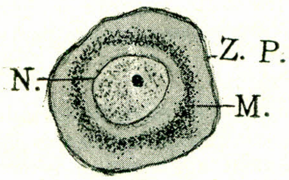

Fig. 3.

layer of mitochondria breaks up into a large number of small patches, which, in the still later stages, travel to the periphery and form more or less a peripheral band. The central cytoplasm is now clear and free from them (Fig. 4). Thus this layer

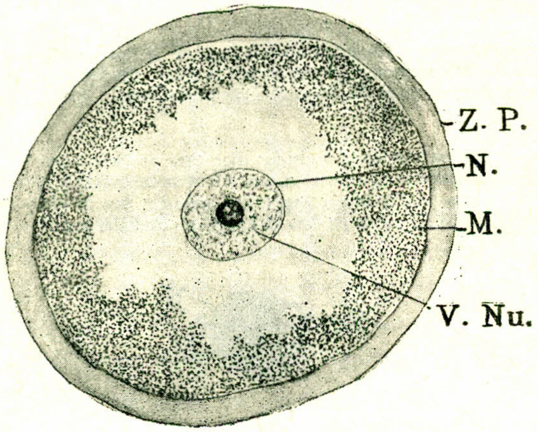

Fig. 4.

shifts from the peri-nuclear to the peripheral region of the egg. Finally, this peripheral band breaks up and the whole oocyte is seen full of small patches of granular mitochondria (Fig. 5).

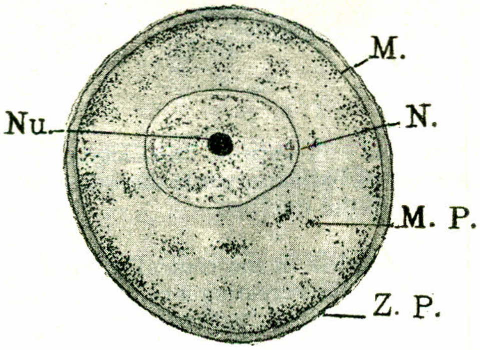

Fig. 5.

It has not been possible to determine the function of mitochondria. Van-der-Stricht[1] reports that in mammals, mitochondria give rise to fatty yolk, but in the animal under investigation, the mitochondria appear to play no part yolk in fatty yolk formation.

Canis familiaris.—The mitochondria behave in the same manner as the Golgi apparatus (Yosufzai).[2–4] The mitochondrial aggregation is at first at the juxta-nuclear position (Fig. 6). Sometimes two

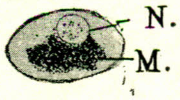

Fig. 6.

patches of granular mitochondria are seen at the opposite poles of the nucleus. Such oocytes are found situated below the germinal epithelium, and being young are devoid of follicle cells.

In the oocytes of slightly bigger size the mitochondrial element is seen concentrated at the yolk-nucleus position. This concentration may homologised with the yolk-nucleus of Balbiani (Fig. 7). In some oocytes it is noticed that mitochondria are concentrated in the yolk-nucleus area as well as few scattered in cytoplasm.

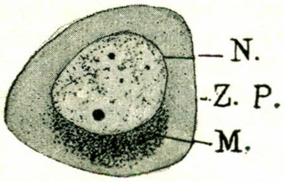

Fig. 7.

The mitochondria increase in number so much on one side that the nucleus is pushed away from the centre to occupy an eccentric position. This condition persists for a long time till the graafian follicle is estáblished in the form of several layers of cells (Fig. 8), while in the initial stages the

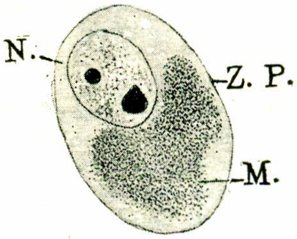

Fig. 8.

mitochondria are granular, in advanced oocytes they become finer and dust like. Soon the cap of dust-like mitochondria disintegrates and the mitochondria are seen uniformly distributed in the whole cytoplasm. In completely mature oocytes however, one can see dust-like and granular mitochondria in the cytoplasm and also a peripheral concentration of it in the form of a ring (Fig. 9).

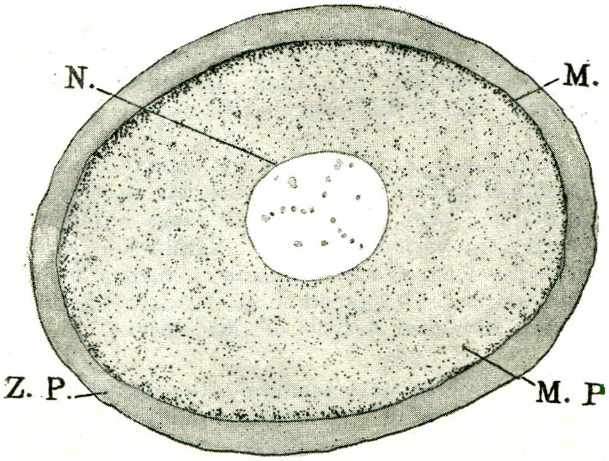

Fig. 9.

Van-der-Stricht[1] described yolk formation from mitochondria. The animal under investigation does not show any trace of albuminous yolk.

Discussion

The mitochondria were discovered by Altmann[5] in 1890 while working on the relation of the alimentary organs of certain animals and were termed bioblasts. In 1902, they were nomenclatured as mitochondria by Benda[6] but many described it as chondriosome. Some workers consider them as symbiotic bacteria. Cytologists have ever since been engaged in describing their polymorphic nature and universal occurrence, but the dispute egardeng their origin remains unsolved so far.

Van-der-Stricht[1] wrote a monograph in which he dealt with this structure. To quote Cowdry,[7] "During the period of growth of the oocyte the mitochondria are found to lie in fairly dense mass, concentrated about the vitelline body (Yolk-nucleus). As the oocyte grows larger, there comes a period during which the mitochondria appear to move away from the centrosomal region and become scattered throughout the cytoplasm". Van-der Stricht[1] termed this stage as "the stage of disaggregation of the mitochondrial layer". Levi[8] agrees with Van-der-Stricht as regards the position and distribution of the mitochondria in the mammalian ovum. Kingery[9] observed granular form of mitochondria in the mouse ovum. The mitochondria are situated round the nuclei of the germinal epithelial cells. When the oocyte advances in age, the mitochondria come to lie at one pole of the nucleus in the form of a crescent-shaped mass; later on, they distribute themselves uniformly in the ooplasm as granules.

Lams and Doorme[10] observed granular mitochondria in groups or scattered singly throughout the ooplasm in the mouse. In the ovum, which was undergoing maturation, he observed mitochondria in several compact masses distributed throughout the cytoplasm.

Gresson[11] based his results on Cajal's uranium nitrate and silver nitrate techniques. He observed the presence of mitochondria as small granules of a golden-brown colour, side by side with the Golgi material. In the older oocytes, having a single-layered follicle wall, he observed mitochondria in large numbers in the vicinity of nucleus and Golgi apparatus and sparingly scattered in the general ooplasm. In the more advanced eggs they are seen evenly distributed, which later collect into clumps leaving the intervening ooplasm free.

Clement[12] observed mitochondria in the young oocytes of squirrel at the juxta-nuclear position. The next stage as reported by her is the formation of a cap at one pole of the nucleus in the form of a concentrated mass, which later on gives rise to perinuclear and medullary rings. In the mature oocytes mitochondria are seen distributed in the form of patches.

According to Beams and King[13] the mitochondria are granular in nature in the developing oocytes of guinea pig. Except for a little concentration in the yolk-nucleus area they are evenly distributed in the cytoplasm.

Aykroyd[14] seems to be in agreement with Van-der-Stricht.[1] In the young oocytes of man, she observed mitochondria concentrated in the form of cresent-shaped structure at the side of the uncleus. This she calls as "Pallial layers or couche Vitellogene" of Van-der Stricht. In addition to this concentration, she noticed a few mitochondria scattered in the cytoplasm. In the following stage the pallial layer breaks down and the mitochondria arrange themselves in a perinuclear fashion. From this perinuclear condition the mitochondria are dispersed into the ooplasm.

Abbreviations used in the Figs:

F. C., Follicle cell; M., Mitochondria; M.P., Mitochondrial patch; N., Nucleus; Nu, Nucleolus; V. Nu., Vacuolated nucleolus; Z. P., Zona pellucida.

The author's own observations reveal that the mitochondria in the initial stages lie in the form of a dense crescent-shaped mass at one pole of the nucleus. Later on this stage gives rise to various stages as reported in the obsrvations. The presence of mitochondria in patches as observed both in monkey and dog was also recorded by Clement[12] Lams and Doorme.[10] As regards the position and arrangement of mitochondria, Kingery[9] on the one hand, observed the mitochondrial crescent-shaped mass in the mouse ovum, but on the other hand, Lams and Doorme[10] and Gresson[11] do not report any such arrangement. It is difficult, therefore, to determine which of the two observations is correct.

Most of the workers on the oogenesis of mammals have described the presence of mitochondrial crescent-shaped mass and the present work confirms these results.

Lams and Doorme[10] reported two typesof mitochondria, namely (1) large granules which were observed, collected into groups or scattered singly in the cytoplasm and (2) small granules which in some cases were arranged in short chains.

Gresson[11] considers large granules to be Golgi bodies. According to him, "Golgi granules and rods of the late ova are slightly smaller than those of the ovarian oocytes and apparently correspond to the large type of mitochondria of Lams and Doorme".[10] The author does not subscribe to the view held by Gresson in the light of his own observations. The presence of large mitochondrial granules is seen in the oocytes of the animals under investigation, which supports the observations of Lams and Doorme.[10]

Beams and King,[13] while working on the oogenesis of guinea-pig have missed all earlier mitochondrial stages as are reported by Clement,[12] Aykroyd[14] and others and also by the present author.

Aykroyd[14] could not discover the presence of mitochondrial patches. She considers Van-der-Stricht's[1] observations faulty, but her statement cannot be justified. Clement,[12] Lams and Doorme[10] and the present author have observed such mitochondrial patches. According to Aykroyd,[14] "The structures that Van-der Stricht figures may therefore in some cases be due to the action of the acetic acid in Benda's fixative, which attacks the mitochondria, and so a true picture is not given." This argument of Aykroyd[14] loses weight because the present observations were based on the use of Regaud-Tupa which has no acetic acid."

Now coming to the form of mitochondria various forms have been recorded. The mitochondria may be rod-like, filamentous, granular, straight, curved, twisted and dumbell shaped.

Jacquiart[15] has described the zig-zag filaments of mitochondria. The mitochondria in granular form have been reported by a large number of workers in various animals. Narain[16] observed granular form of mitochondria in earlier oocytes and dumb-bell shaped and beaded filaments in the advanced oocytes of *Ophiocephalus*.

According to Bhattacharya[17] the mitochondria are in the form of very small granules which later on may form patches in the eggs of tortoises. Loyez[18] observed granular form of mitochondria in the oocytes of man. Clement,[12] Aykroyd,[14] Gresson,[9] Beams and King[13] have described granular form of mitochondria in various mammals. The present author has observed granular form of mitochondria in the case of monkey and dog, and, therefore, the findings are in confirmity with the observations of other workers on mammalian oogenesis.

As regards the origin of mitochondria, there again is found a controversy among workers. Some believe that the mitochondrial granules are transmitted from generation to generation as such. Gatenby[19] for instance, reported the presence of mitochondria in the undifferentiated germ cells in *Dytiscus* and Bhattacharya[17] in Indian reptiles. The other school believes that the mitochondria are entirely absent in early oocytes and later on are formed *denovo* in the cytoplasm. Among such workers the names of Gardner[20] and Nath[21] in *Lithobius* may be mentioned.

The author's own findings are in agreement with those of Bhattacharya, Gatenby and others who believe in the pre-existence of mitochondria. In the case of monkey, when eggs divide, the mitochondria are also sorted out. This shows that the mitochondria pre-exist.

Heberer[22] in *Encalanus* reported that the nucleoli of the oogonia enter into the cytoplasm and condense there, giving rise to cap-like yolk-nucleus at the juxta-nuclear position, and in that region the mitochondria are differentiated.

As regards the function of mitochondria, there are interesting and controversial findings among the workers. Altmann[5] considered them as vital units suspended in long living matrix but the idea was soon given up by other workers. Benda[6] and Meves[23] suggested that they played an important part in heredity. Nassonov[24] and Ludford[25] hold that mitochondria also play some part in the origin of secrotary granules.

According to Kingsbury[26] "the mitochondria are phospholids containing unsaturated fatty acids with ethyledene groups and therefore well adapted to function in oxidation and reduction". Gatenby[19] in his work on the amphibian oogenesis remarks, "It seems natural to conclude that yolk grains are formed from the metamorphosed mito chondria as it is believed to occur in other animals". Ludford,[25] Bhattacharya,[17] Nath[21] and many others have observed that the mitochondria are similar to plastids and elaborate complex material from the surrounding ooplasm.

Harvey[27,28] is of the opinion that the reare three ways in which mitochondria play their part in yolk formation. Firstly, the yolk results by the direct chemical transformation of mitochonria. Secondly, yolk is formed inside the mitochondria, and the material for yolk formation is supplied by the cytoplasm. Lastly, molecules, mainly of protein composition, are deposited among the mitochondrial molecules and the resultant complex is called yolk.

Van-der-Stricht[1] is the only worker who brought forward an altogether new idea. According to him, the second layer of mitochondria of the yolk-nucleus retains its primitive aspect and it is this that mainly elaborates the first deutoplasmic fatty granules.

According to Gresson[11] and Harvey[27,28] "protein yolk arises under the influence of the mitochondria and Golgi bodies from material derived from plasmosomes, ground cytoplasm and external sources. If this be true, it is probable that the mitochondria or Golgi elements or both play some part in the formation of the scanty yolk of the mouse ovum".

The author has not observed mitochondria playing any role in the elaboration of fatty or mitochondrial yolk in the ovum of dog or monkey. His findings are in agreement with those of Clement,[12] Beam and King[13] and Aykroyd.[14] There is absolutely no indication that the mitochondria are responsible for fatty yolk formation as reported by Van-der-Stricht in 1923.[1]

Chevremont[29] reported that cytochemical studies of chick fibroblasts show that mitochondria accumulate Desoxyribonucleic acid which is synthesized in the cytoplasm and transported to the nucleus by the mitochondria. In 1963, S. Nass and M. Nass[30,31] stated, "If the presumed DNA—containing material in the mitochondria has genetic properties similar to those reported for other DNA molecules, then the results also support the genetic view that mitochondria are mutable and possess some degree of reproductive autonomy". The present author agrees with the views of these workers.

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