

# Contribution to the Study of Mitochondria

By

**Syunzô TAKAGI**

(Department of Physiology, Nagoya Medical College)

---

*With 6 Text-figures*

---

(Received February 21, 1939)

---

## Contents

	Page
I. Introduction . . . . .	167
II. Outlines and the Results of the Author's Own Studies . . . . .	169
III. On the Mitochondria as the Formed Constituents of Protoplasm . . . . .	176
1. On the occurrence of the mitochondria . . . . .	176
2. On the shape and size of the mitochondria . . . . .	178
3. On the localization and arrangement of the mitochondria in the cell . . . . .	183
4. On the quantity and changes in quantity of the mitochondria of the cell . . . . .	187
IV. On the Nature and Functional Significance of the Mitochondria . . . . .	193
V. Summary . . . . .	200

## I. Introduction

In 1927 the present writer began his study on the mitochondrial problem at the suggestion of Prof. S. KATSUNUMA of Nagoya Medical College, the behavior of mitochondria of the toad during hibernation and inanition being selected as the subject. Since then under the direction of Prof. Yô K. OKADA of Kyoto Imperial University he has continued his efforts to elucidate the problem mainly from the standpoint of general biology. To these gentlemen the writer is particularly indebted in various ways. Not a few works on individual subjects had already been published in various journals of biology and medicine. It is the aim of the present paper to bring forward some important conclusions on the biology of the mitochondria obtained by the writer from his own works, from general theoretical considerations, and from critical survey of the literature on the subject.

Although various related objectives were sought in each of his studies, most of them were intended primarily for the purpose of throwing light on one or both of the following questions: 1. Is there any definite relation between qualitative or quantitative features of the mitochondria and levels

of functional activity of the cell? 2. What relation exists between the mitochondria and the ontogenetic history of the cell? 3. Are there no peculiarities to be found about mitochondria in animals low in the phylogenetic scale? 4. Are there any direct relations between the mitochondria and visible differentiations of the cytoplasm? Outlines and the main results of these works will be given briefly in Chap. II. Some of the results of investigation that have remained until now unpublished will be included in Chap. III.

Through his studies covering many years, the author has become convinced of the fact that there exist some laws or general rules about the morphological features, i. e. the size, shape and arrangement, and the quantity of the mitochondria in the cell. Although ignored by most of the investigators, this is what ought to be expected of cytoplasmic constituents of such universal occurrence as these. Any theoretical considerations on the nature or functional significance of the mitochondria must be based upon recognition of these rules. It may rather be said that the studies on the mitochondrial problem should be directed first to the establishment of these rules and then proceed to more fundamental questions. To the fact that this natural order of things has not been observed is due part of the present chaos of the mitochondrial problem. It is for this reason that some important rules governing the morphological and quantitative aspects of the mitochondria are dealt with in Chap. III of the present paper.

The most important conclusions arrived at by the author are as follows. 1. The mitochondrial content of the normal cell does not undergo quantitative changes to any marked degree during the functional cycle of the latter. On the contrary, it does increase or decrease during the ontogenetic history, i. e. differentiation or senescence, of the cell, but it is accompanied here with simultaneous increase or decrease respectively in quantity of the cytoplasm. 2. Mitochondria may be regarded as constituting special phases of the hyaloplasm in which they are embedded. 3. Although the functional significance of the mitochondria most probably consists in surface catalysis, the extent to which they are utilized in this sense has no connection with the fact of their existence. As to these points the reader is referred to the last section in Chap. III and to Chap. IV.

The author's study on the mitochondrial problem was begun at the Biological Laboratory of Aichi Medical College and is now continued at the Department of Physiology of Nagoya Medical College. But some of his investigations were conducted and the main ideas at the base of his arguments in the present paper arrived at and published in Japanese, when during the years 1933-1936 he was a lecturer in the Zoological Institute of Kyoto Imperial University.

#### Acknowledgement

It is a pleasure to record here my debt of gratitude to the learned gentlemen mentioned above, Prof. S. KATSUNUMA of Nagoya Medical College and Prof. Yô. K. OKADA of Kyoto Imperial University for their kind sug-

gestions and encouragement. To Prof. OKADA I am especially indebted for his kindness in taking time from his busy life to revise the manuscript. The author's sincere thanks are due, also, to Prof. YAS KUNO, director of this department, to Prof. T. KAWAMURA and Prof. T. KOMAI of Kyoto Imperial University, and to Prof. K. HUKUDA of Tokyo Imperial University for their cordial support of his investigation.

## II. Outlines and the results of the author's own studies

In the following are given brief outlines and conclusions of the author's works published since 1927. These were in German, English or Japanese. For the name and the number of volume of the journals in which the original papers appeared the reader is referred to the list of literature at the end of this paper.

I. On the behavior of mitochondria in the various organs of the toad during hibernation and inanition. (In German). 1929 a.

This investigation was made during the period lasting from August 1927 to December of the next year with *Bufo vulgaris formosus* as the material. A total of 45 individuals was used. 38 of these were employed for study on the seasonal changes of the mitochondria in the various organs, namely liver, pancreas, stomach, intestine, heart, kidney, muscle, oviduct and uterus, and the remainder for study on the changes of the mitochondria owing to inanition during the summer season, the purpose of the latter series of experiment being to make comparisons between hibernation and inanition, two apparently similar phenomena, on cytological grounds. Fixation was in REGAUD's fluid, postosmication, staining after BENSLEY's method published by COWDRY.

The digestive organs and the muscle are in summer at the zenith of their activity, but in winter their function falls or wholly stops. This phenomenon finds its cytological parallel in that the mitochondria show changes in morphology or topography. In the liver cells during the summer season only filamentous mitochondria are found, but in typical hibernation toads they are mostly rod-shaped or granular with a few filamentous ones. In the pancreatic cells, also, granular forms increase in winter, whereas filamentous ones markedly decrease. The mitochondria of columnar epithelial cells of the intestine become in winter indistinct as to their individuality. Changes occurring in the stomach and the muscle are inconspicuous. Though the fundic gland cells of the stomach are found in summer to be rich in filamentous mitochondria, they are provided in hibernation with granular and filamentous ones, the latter being shorter and thicker than those in summer. The muscle (the sartorius) consists, as it does in all the vertebrates, of two kinds of muscle fibres. One of them, which corresponds to the red muscle fibre of other vertebrates, shows no seasonal change of mitochondria, while in the other, which corresponds to the pale muscle fibre, those which are in summer widely distributed between the bundles of myofibrils tend in winter to form small clusters. No seasonal changes of the mitochondria

have been observed in the heart muscle, nor in the kidney so far as the glandular and the striped canal of the urinary tubules are concerned. In the oviduct and the uterus the epithelium becomes desquamated after the egg laying and regenerated afterwards. In the young cells abundant mitochondria are found.

In inanition conspicuous changes occur in the liver and the intestine. In the hepatic cells mitochondria remain filamentous, but tend, in marked contrast to those of normal summer toads, to be localized in the basal part of the cell (after inanition of 27 to 60 days). In the columnar epithelial cells of the intestine, mitochondria in the middle and the basal portions of the cell markedly increase, while those in the portion near the lumen decrease. Observations made on the other organs show nothing very different from those on normal summer toads. Thus, the inanition and the hibernation are two entirely different things in view of the behaviour of mitochondria, a fact which is also evident from the physiology of metabolism.

2. On the mitochondria in the electric organ of *Narke japonica*. (In German). 1929 b.

Two adult male individuals of the electric fish were used as material. Small pieces of the electric organ were fixed in REGAUD's fixative and the sections were stained after ALTMANN-KULL.

The fish possesses a pair of electric organs in the branchial region, each of which consists of about 200 electric pillars arranged dorsoventrally, each of which, again, is made up of a large number of electric plates arranged transversally. Between the electric plates is a thin layer of connective tissue, in which small blood vessels and nerve fibres are embedded. Each electric plate may be considered to be a single cell, though it contains many rounded nuclei. It is divided, though not very distinctly, into a dorsal and a ventral layer by delicate lamellae. The nuclei are contained in the dorsal layer.

The mitochondria in the electric plate are mostly filamentous. They are present in both the dorsal and the ventral layers, but they are distributed only close to the line of boundary delimiting the two layers and immediately around the nucleus. It can be seen that the electric organ, of which the main mass consists of electric plates, is not very rich in mitochondria.

3. On the mitochondria in the PURKINJE cells of the conducting system of the heart. (In German). 1929 c.

Small pieces of tissue were taken from the bundle of HIS and its right and left branches. Hearts of the ox and the pig were employed while still warm. REGAUD's fixative and the stain according to ALTMANN-KULL were used.

In the PURKINJE cells mitochondria, filamentous in form, are widely distributed in the cytoplasm. They are, however, especially abundant close around the nucleus and near the periphery of the cell. In the PURKINJE cells are numerous myofibrils running generally in small bundles. Although the mitochondrial filaments often run intertwining with these, they are never in direct continuity with the myofibrils.

4. Studies on the differentiation and the mitochondrial content of blood cells. *Haematopoiesis in the yolk-sac of chick embryo.* (In German). 1931.

Mitochondria in developing blood cells in the yolk-sac of chick embryo were studied and their changes accompanying cellular differentiation were followed. Small pieces of the yolk-sac in different stages of development were fixed in MEVES' fluid and the sections were stained after ALTMANN-KULL. Haematopoiesis was divided, after the pattern of KIYONO and KATSUNUMA, into three stadia, the first of these including only the primitive blood cells, the second the primitive erythroblasts and erythrocytes, lymphoid cells, histioid cells and primitive endothelial cells, and the third the definitive erythroblasts and erythrocytes, lymphocytes, thrombocytes, histiocytes, myeloblasts and myelocytes, and definitive endothels. Descriptions of the mitochondria of these cells were given, for the details of which reference should be made to the original paper. Parts of the results will, however, be given in the next chapter when the relation between the morphological and quantitative aspects of the mitochondria and the cellular differentiation will be dealt with (see p. 179, p. 190, and fig. 6).

In conclusion it may be said that the blood cells in the first stadium are not yet differentiated with respect to the mitochondria they contain; those in the second stadium are differentiated to some extent respecting the quantity and the shape of the mitochondria; and those in the third stadium show marked differentiations. Differentiations of the blood cells with respect to the other qualities, such as their content in oxidase granules, vitality and supravitality stainable granules, and phagocytosis, have already been established by other workers (KIYONO and KATSUNUMA). Changes of the mitochondria do not go parallel with any of these qualities. It is also noted (1) that considerable growth in length of the mitochondria occurs in the erythroblasts at the time of haemoglobin formation, and (2) that intimate topographical relations exist in the myelocytes between the primordial leucocytic granules and the mitochondria. But this does not necessarily lend support to the view that the mitochondria are direct forerunners of haemoglobin or of leucocytic granules.

5. On the behavior of mitochondria in the mitotic cell division of younger blood cells. (In German). 1932 a.

During the study above outlined of the haematopoiesis in the yolk-sac of chick embryo, younger blood cells were often found undergoing mitotic cell divisions. Mitochondria of a dividing cell are divided into two apparently equal groups as early as in the metaphase. They are distributed in this stage around the poles and the mitotic spindle, but are absent on the equatorial plane, thus constituting two distinct groups. They do not enter, as is well known, into the body of the spindle. When during the anaphase, the daughter chromosomes move apart and pass toward opposite poles of the spindle, mitochondria come in turn to be localized between the resulting chromosome groups. Thus, in the late anaphase and the telophase none of them remain around the poles. When two daughter cells have just

separated, mitochondria are all on the opposite side of the newly formed nucleus.

This behavior of mitochondria during the cell division may probably be best explained by the growth of astral rays in the metaphase, which induce cytoplasm with mitochondria in it to flow toward and accumulate around the poles, and by the disappearance of astral rays in the ensuing anaphase with the elongation of the mitotic spindle, which causes backflow of the cytoplasm with mitochondria toward the equator. Confining ourselves to the vertebrates, it is curious to find that this regular behavior of mitochondria during the cell division has never been reported by the students of mitochondria, even by LEWIS and LEWIS who worked on tissue culture cells. This is probably due to the fact that the blood cells which served us as the material are allowed free cytoplasmic streaming, which, however, may not be the case with cells forming firm tissues. Such considerations lead to the assumption that the behavior of mitochondria in mitotic cell divisions is most typically represented in the case of our present report. In reality, it was found that, in dividing cells of loose connective tissue of chick embryo at the second day of incubation, mitochondria behaved themselves in essentially the same manner as in the dividing blood cells. (UETANI in 1938 recorded that the mitochondria in dividing bone marrow cells of man behaved in just the same way as in our case).

6. Studies on mitochondria. (A report made at the 7th meeting of the Japanese Zoological Congress). (In Japanese). 1932 b.

This report included besides the others, two observations which have remained otherwise unpublished: The pecten, which is a structure peculiar to the eye-ball of the bird, is made up of glia cells and numerous small blood vessels. The glia cell contains, besides a number of pigment granules of variable size, a considerable number of mitochondria in the form of rods or short filaments. They are more numerous than elsewhere around the nucleus and at the periphery of the cell toward the vitreous humour. Transformation of mitochondria into pigment granules can not be observed. In the endothelial cells lining the blood vessels are found mitochondria which are very thin and in the form of short filaments. Pectens from chickens and adults of the domestic fowl were used as the material.

Mitochondria were studied in various types of amoeba. An amoeba of *Vahlkampfia* type found in a hay infusion has filamentous mitochondria. Such forms of mitochondria also occur in an amoeba of *radiosa*-type. An amoeba of *proteus*-type is provided, on the other hand, with only granular forms of mitochondria. The same is true of an amoeba of *limax*-type. Topographically there exists no particularly intimate relation between the mitochondria on the one hand and the nucleus, the food vacuole or the contractile vacuole on the other. Mitochondria are not present in the ectoplasm. Amoebae were fixed on slides merely by the action of the fixatives and mitochondria stained with ALTMANN'S acid fuchsin.

7. On the mitochondria in *Dicyema*. (In Japanese, with a summary in German). 1932 c.

As the animal is small and consists only of a small number of cells, mitochondrial technique (MEVES' or CHAMPY'S fixative, ALTMANN-KULL'S stain) can be applied successfully to a number of individuals collected in a centrifuge tube, preparations *in toto* being made.

The body of *Dicyema* (an agamont) consists of pole cells, outer cells and an axial cell. Mitochondria are more abundant in the first in relation to the size of cell than in the other two kinds of cells. In these they are present in moderate quantities. In the pole cells, they are all granular and are more numerous around the nucleus and at the side of the cell toward the exterior. The different pole cells do not differ much in respect to their mitochondrial content. Mitochondria in the outer cells are mostly granular with occasional rod-like ones, and are smaller in diameter than those of the pole cells. They are uniformly distributed in the cytoplasm, though they are somewhat more numerous at the periphery of the cell toward the exterior. The axial cell contains rod-like mitochondria with a few granular and short filamentous ones. Their diameter is almost the same as those of the outer cell. They appear at first sight to be unevenly distributed in the cytoplasm, but this is evidently due to the vacuolated condition of the latter.

8. Some observations on the mitochondria in the cyclostome, *Entosphenus japonicus* (MARTENS). (In German). 1933 a.

Mitochondria of the cells of the stratified epithelium of the gill-pouch and the gill-lamella are described and attention is paid to the relation between the aging of cells and the change in thickness of the mitochondria (fig. 2). It was found also that, although the liver of this animal lacks the function of secreting bile, the hepatic cell is no less rich in mitochondria in relation to the volume of cytoplasm than that of any other vertebrate. No evidence is obtained, further, in favour of the view that the hepatic cell mitochondria participate in the formation of fat droplets. The intestinal epithelium consists of cilia-bearing cylindrical cells and a few unicellular glands with basophilic secretion granules. Mitochondria are mostly filamentous in both types of cells. Their distribution and orientation in the cilia-bearing cylindrical cells are strikingly similar to those in the absorbing cylindrical cells of the intestinal epithelium of other vertebrates and probably suggest a like function on the part of the former (fig. 4). The muscle is made up, as in higher vertebrates, of red and pale muscle fibres, the former being richer in sarcoplasm than the latter. Mitochondria are mostly in the form of short filaments and are more abundant in the red fibre than in the pale. They are arranged end to end in a single file when they are embedded in the narrow sarcoplasm between bundles of myofibrils. This is evidently a forced arrangement, for those which are embedded in the wider sarcoplasm under the sarcolemma are arranged quite at random. Furthermore, mitochondria in the arachnoideal cells, cardiac muscle, tela chorioidea and kidney are described. REGAUD'S fixative, postosmication, ALTMANN-KULL'S stain.

9. Mitochondria in the luminous organs of *Watasenia scintillans* (BERRY). (In English). 1933 b.

The cephalopod is possessed of three kinds of luminous organs (cutaneous, ocular and brachial). Each of these has in the seemingly important part of its tissue, some granules, so-called photogenic granules, of which the nature and function remain unknown. (The latter upon subsequent study by OKADA, SUGINO and the writer proved to be crystalloids of protein). The present aim was to examine the mitochondria in various tissues of the luminous organs and to establish, if possible, the relationship between the mitochondria and the photogenic granules. It was shown as the result that mitochondria exist in these organs independent of the photogenic granules, that they are not or only rarely present in the neighbourhood of the latter, and that they are abundantly existent in apparently unimportant regions of the organs, namely the connective tissue and the epidermis. Incidentally it may be said that this study together with others led the author to believe that the presence and the quantity of the mitochondria in the cell depend not so much on the functional activity of the latter as on the presence and the quantity respectively of morphologically and, in some sense, chemically undifferentiated protoplasm. Fixation with ZENKER-formol-osmium, staining with ALTMANN'S acid fuchsin and Lichtgrün or with HEIDENHAIN'S iron-hematoxylin and dilute acid fuchsin.

10. Mitochondria in the luminous organs of *Luciola cruciata* MOTSCHULSKY. (In English). 1934 a.

The luminous organs of the adult and larval insect were studied. Cytological technique was almost the same as in the preceding. In the luminous organ of the adult, the so-called photogenic granules in the light cells are minute, variable in size, and irregular, though in general round. They are abundant and uniformly distributed in the cytoplasm. Mitochondria are mostly filamentous and curved, and are uniformly distributed throughout the cytoplasm. The photogenic granules of the larval insect, though of variable size, are far larger than those of the adult. Most of them are oval and they are uniformly distributed throughout the cytoplasm. Mitochondria of the cells are filamentous, but branching is not rare among them. It is noteworthy that filamentous mitochondria in the light cells of the larval as well as adult organs run quite at random, i. e. they do not exhibit definite orientation toward the nucleus, cell boundaries or tracheal system. In the light cells of both the adult and the larval insects the photogenic granules are quite independent of the mitochondria. Nevertheless, we find here and there mitochondria somewhat thickened at the ends or at the intermediate portion. These thickenings are probably due to the deformation of mitochondrial substance by the action of the fixative. But there is some reason to suppose that they represent an initial stage of the development of photogenic granules.

11. On the formation of secretion in the luminous organ of *Cypridina hilgendorfi* MÜLLER, with special reference to the mitochondria. (In German). 1936 a.

The luminous organ of the animal is a compound gland composed of



four types of large unicellular glands. It was the aim of this investigation to study the fine structure of these four types of glandular cells, their mode of formation of secretion granules and the relation, if any, between these and the mitochondria of the cells. The mode of formation of secretion granules differs according to the type of cell and is of interest in view of comparative physiology of glandular cells, into which, however, this is not the place to enter. In all the four types of cells, mitochondria, mostly granular, are found uniformly distributed in the basal protoplasmic region containing the nucleus. There is no evidence that younger stages of the secretion granules are directly derived from the mitochondria. Fixation with ZENKER-formol-osmium, staining with HEIDENHAIN'S iron-hematoxylin.

12. Cytoplasmic inclusions of the erythrocyte of *Triturus pyrrhogaster* (BOIE). (In Japanese). 1936 b.

Mitochondria, GOLGI material and neutral red granules in the erythrocyte of the Japanese common newt were described. Beautiful stain of the mitochondria with Janus green B was obtained with SABIN'S supravital technique, but they could hardly be detected in the blood smear treated with usual mitochondrial fixatives and stains. Mitochondria in various shapes, i. e. filaments, rods and granules, occur in the cytoplasm. They are more numerous in younger erythrocytes than in the full-grown ones.

13. A quantitative study of mitochondria in some ciliates. (In Japanese). 1939.

Some ciliates possess mitochondria, which are generally rod-like in shape and approximately equal in size, and furnish favorable objects for quantitative study. The cytoplasm of *Spirostomum ambiguum* and *Blepharisma lateritium* includes, in addition to the contractile and food vacuoles, a large number of fluid vacuoles of variable size. Avoiding as far as possible these kinds of vacuoles, enumeration was made of the mitochondria contained in a definite volume of the cytoplasm. Those 10 individuals of each of the two species were chosen, which gave serial sections along the longitudinal axis of the body. 3 contiguous sections through the middle of the body were used of each individual, and in each section enumeration of the mitochondria was made at 4 different localities in *Spirostomum* and at 3 in *Blepharisma*. Thus, the largest number of mitochondria contained in 660 cubic microns of the cytoplasm was determined for each individual out of 12 enumerations in the former and 9 in the latter. The numerical data in order of magnitude for the 10 individuals of each of the two species were as follows.

*Spirostomum*: 38, 43, 50, 57, 57, 61, 62, 73, 75, 78.

*Blepharisma*: 51, 55, 55, 56, 60, 61, 70, 76, 77, 83.

Only the largest of the numbers thus obtained may be so significant as to represent approximately the number of mitochondria contained in the definite volume of cytoplasm in the narrow sense of this term, for a varying number of small fluid vacuoles inevitably comes to be included in that volume of the cytoplasm where enumeration is made, requiring correction

by reduction in the number. Thus, the number of mitochondria in a definite volume of cytoplasm is known to be practically equal between the two species of the ciliate. If 80 mitochondria are considered to be contained in 660 cubic microns of the cytoplasm, a little computation shows that this is equal to the density of 121 million per cubic milimeter. This is not very different from THURLOW's ('17) data of 178-284 million per cubic milimeter about the nerve cells of various cerebral ganglia of the mouse and also from RASMUSSEN's ('20) of 186-354 million about the nerve cells of the central nervous system and spinal ganglia of the woodchuck.

14. A treatise on mitochondria. I. On the quantity and changes in quantity of the mitochondria of the cell. (In Japanese). 1934 b.

15. A treatise on mitochondria. II. On the form and changes in form of the mitochondria. (In Japanese). 1934 c.

16. A treatise on mitochondria. III. On the arrangement of mitochondria in the cell. (In Japanese). 1935.

In these three papers considerations and discussions on diverse lines were made by the author for the purpose of establishing general rules to which the mitochondria are subject. The main line of argument is the same as will appear in the next chapter of the present paper.

### III. On the mitochondria as the formed constituents of protoplasm

#### 1. On the occurrence of the mitochondria.

Mitochondria have been studied in animals and plants belonging to nearly all of the larger natural groups. N. H. COWDRY asserted as early as 1917 that "in all forms of animals, from amoeba to man, which have been investigated with adequate methods of technique, they occur without exception." Newer data which had been accumulated since then contributed much to our general conviction of their universal occurrence in the animal kingdom. It seemed unfortunate, however, for making a generalization, that some of the relatively important groups of animals remained untouched. Animals belonging to Mesozoa had never been studied till the author described mitochondria in the main types of cells in *Dicyema* (p. 172). The Mesozoa are generally acknowledged to be not an animal group of specific organization, but a heterogeneous group consisting of various degenerated forms. It was thus actually proved that such degeneration in organization is not attended with deprivation of mitochondria in the cell. The Cyclostomata, the lowest class of Vertebrata, also, had never been the subject of study by students of the mitochondrial problem. Mitochondria in the various organs of *Entosphenus* were studied by the author and shown to be similar in general appearance to those in other vertebrates (p. 173). This holds true not only of the liver, the intestine, the kidney etc., but also of those tissues which are unique to the Cyclostomata, namely the arachnoideal tissue and the epithelium of the gill-sac. The liver and the intestine of this animal differ in function somewhat from the corresponding organs of other ver-

tebrates,<sup>1)</sup> but the form and orientation of the mitochondria in the cell are largely the same. Thus it was shown that mitochondria are not only present in the lowest representative of the Vertebrata, but also they much resemble those in the higher. We are now in a situation to say with more self-confidence than ever that mitochondria exist, without exception, in all the animals.

Mitochondria, however, do not occur in all the animal cells. It is well known that they are not present in the erythrocytes of mammals including man (CIACCIO '13, COWDRY '14 a). It was observed by the present writer that mitochondria in the form of filaments are abundant in the erythroblasts of the domestic fowl, but they are not detected in the erythrocytes. Existence of mitochondria, however, could clearly be demonstrated in the erythrocytes of the Japanese common newt by supravital staining with Janus green B (p. 175). It seems therefore justifiable to conceive that the process of erythropoiesis in the higher vertebrates is accompanied with a profound chemical or physicochemical change of the protoplasm which is incompatible with further maintenance of mitochondria. It was also reported that although mitochondria are abundant in cells of the stratum germinativum of the skin, they are almost or practically absent in the stratum granulosum and lucidum and completely absent in the stratum corneum (REGAUD and FAVRE '12, LUDFORD '24, COWDRY '34). The disappearance of mitochondria in this case, i. e. in accompaniment with advancing keratinization of living cells, may, in all likelihood, be interpreted in the same way as in the above case of erythropoiesis in the higher vertebrates. As will be shown later, changes in the quantity of mitochondria occur most indubitably in the process of cellular differentiation and senescence, leading in extreme cases as in the above two examples, to their complete disappearance.

It is necessary, however, to bear in mind that cells such as those of the stratum corneum of the skin or the erythrocytes of the higher vertebrates are, in reality, dead or nearly dead cells. Through years of study the present writer has become convinced that living cells wholly devoid of mitochondria, if they ever exist, must be of rare occurrence. But in a survey of literature, authors are often met with who report absence of mitochondria in apparently healthy cells. It seems that they have not sufficient knowledge of the difficult details of mitochondrial technique. It must be realized that the supravital staining of mitochondria with Janus green B is sometimes very difficult and in some cases even impossible, as was experienced by GUILLIERMOND ('18) and the writer himself. In addition, mitochondrial fixatives, when inappropriately chosen for the tissue to be examined, may sometimes lead to complete dissolution of the mitochondria. Hence, in studying those of a given tissue, a variety of fixatives must first be applied and the one which gives the best results chosen, subsequent staining

---

1) The liver does not secrete bile and has no bile-duct in the Cyclostomata. The intestine, in *Entosphenus* at least, shares a function of disposing of worn-out erythrocytes with the branchial epithelium (TAKAGI, S. '34 d).

being a comparatively easy matter. Absence of mitochondria from any particular kind of cell, therefore, cannot be asserted until after all the known methods of mitochondrial technique have led to entirely negative results. The following example may probably serve as an illustration of the situation. In amphibian erythrocytes mitochondria can scarcely be demonstrated by the fixatives and stains usually employed for the purpose. Thus, AVEL ('24) reports, examining a number of preparations, that even no trace of them could be detected in the erythrocytes of *Rana fusca*; and KURASHIGE ('30) arrived at the same results about those of *Megalobatrachus japonicus* with CHAMPY-ALTMANN-KULL preparations in use. But, that they are present in amphibian erythrocytes is beyond question, for they are fully demonstrated by the supravital technique with Janus green B, as was done by DAWSON ('28) in the erythrocytes of *Necturus* and by the present author in those of *Triturus pyrrhogaster* (p. 175). I may mention in passing that judging from their figures and from my own results of study in *Triturus*, part at least of the alleged GOLGI material of AVEL and the greater part of that of KURASHIGE are actually true mitochondria brought to demonstration by over-impregnation with silver or osmium.

## 2. On the shape and size of the mitochondria.

Mitochondria occur in the cytoplasm as minute bodies of various shapes. Granular, rod-like and filamentous ones are of common occurrence. Occasionally filamentous ones are branched. Such shapes as rings attached to a stalk and networks are only rarely met with. The former were observed by the writer in the hepatic cells of *Bufo* and the latter in those of *Triturus*. Elongated forms of mitochondria are always smooth in outline and possess rounded ends, never tapering toward their extremities. This was always true, as far as our observations were concerned, in vitally stained tissues as well as in those which were appropriately fixed and stained. But, it was not necessarily so in those tissues of which the general result of fixation was unsatisfactory. So-called moniliform mitochondria, i. e. granules in chains, have never been observed by the writer either in living tissues or in those which were considered well preserved by fixatives. They were observed by GUILLIERMOND ('27) occurring as transitory structures in living plant cells and they have been repeatedly described by others in fixed animal tissues. It seems to me, nevertheless, to remain still an open question whether they really exist as such in healthy living cells of animals and whether, in fixed cells, they are not artifacts produced by the action of fixatives.

The appearance of mitochondria in a given type of cells is nearly definite. In some types of cells (e. g. certain types of amoebae, the hepatic cells of the mouse) they are granular, in others (e. g. ciliates such as *Spirostomum* and *Blepharisma*) they are rod-like, and in still others (e. g. the erythroblasts of the domestic fowl, the pancreatic cells of the mouse) they are filamentous. In general, however, various shapes of mitochondria occur simultaneously in the same cell, though one shape usually predominates over

the others (e. g. the hepatic cell of *Triturus*, fig. 1). It is a significant fact that the mitochondrial appearance of a given tissue is nearly definite in different individuals of the same animal, if the tissue stands at the same developmental stage in ontogeny and at the same phase of functional activity. This is shown to hold true always when many individuals of the same species are dealt with. It is the fundamental rule upon which all the experimental studies of mitochondria are based. It is conceived by many, however, that the mitochondria are easily changeable in form and some others even hold that they change in form from moment to moment. But this is not the case, at least in healthy animal tissues, as will be shown later (p. 182). Even during the ontogenetic history and the cycle of functional activity of the cell, change in form of the mitochondria is no necessary attendant. But that it occurs in many cases is evident in a large volume of literature and in the writer's own experience. It was shown by him that in younger stages of the erythroblasts of the domestic fowl rod-like and filamentous mitochondria coexist, but in older stages they are almost exclusively filaments in form, and among them markedly long or distinctly branched ones are found (fig. 6). SMITH ('31) reports that in the ontogenetic history of the hepatic cells of the white rat, morphological changes of the mitochondria often take place—from spherules to filaments and from filaments back to spherules—before the mitochondrial configuration of the adult hepatic cell is attained consisting of filaments, rods and spherules. That change in shape of the mitochondria often accompanies the functional cycle of the cell may be illustrated by the following examples. It was revealed through my own study that in the liver cells of the toad during summer days mitochondria are almost all filamentous with a few exceptional ones, whereas in the typical hibernation toad they are rod-like or granular with a few filamentous ones. In the hepatic lobule of the cat, KATER ('31) found that the mitochondria are rod-like to filamentous, the shortest being found in the cells bordering the central vein and the most filamentous being located in the cells at the outer margin of the lobule. If the glycogen-glucose equilibrium is disturbed through injection of insulin, adrenalin, etc., the mitochondria exhibit a tendency toward enspherulation and hypertrophy, which are more emphasized in the cells bordering the central vein. He rightly concluded that the variations in mitochondrial morphology may be interpreted merely as an accompaniment of alterations of general metabolic activity of the liver cells.

E. V. COWDRY ('14 b) first paid attention to the fact that the appearance of mitochondria are much the same in similar tissues of nearly related animals. It was for him by no means a simple matter, even in the different classes of vertebrates, to distinguish the spinal ganglion cells on the basis of morphology of the mitochondria contained. It was found by the present writer that in appearance mitochondria in the liver cells and the absorbing cells of the intestine of the lamprey, the representative of the lowest class of vertebrate, closely resemble those in the corresponding cells

of the higher classes (p. 173). Roughly speaking, the cells of the liver, pancreas, lungs, and other organs possess mitochondria which are alike in nearly related animals. It is obvious, however, that the extent to which this rule holds true varies according to the tissues and the animals to be compared. It is remarkable to find that in the phylum of Protozoa mitochondria in the protoplasm of such ciliates as *Spirostomum*, *Blepharisma* and *Colpoda* are similar in appearance, i. e. they are all rod-like (p. 175). But in different types of amoebae their appearance is not always similar (p. 172, fig. 5).

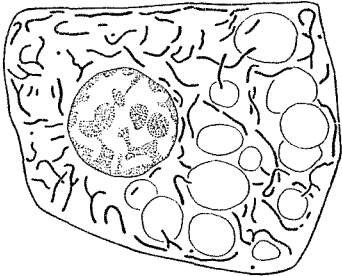


Fig. 1.—Mitochondria with fat droplets in hepatic cell of *Triturus pyrrhogaster*. (After TAKAGI '34 c).

Turning to the size of the mitochondria, it is significant to find that, although they may vary in diameter or in girth in different tissues, they are of approximately the same diameter in any one cell, whether they may appear in the shape of filaments, rods or granules. Even when they consist of various shapes, their diameter is astonishingly uniform (see e. g. fig. 1). This is always true in living cells as well as in those which are well preserved and it represents another fundamental rule of mitochondrial morphology. It was first pointed out and its importance repeatedly emphasized by COWDRY ('18, '24, '26). But, regrettably, it has not received due appreciation. Of course, mitochondria in a cell are not strictly of the same diameter, and a few differences certainly exist. Branched forms of mitochondria may be thicker at the point of bifurcation, and thickenings may also be found on filamentous ones when materials are stored in their interior. Nevertheless, it remains true that all of the individual mitochondria in a cell are nearly definite in diameter. All the observations made by the writer are in accord with this proposition.

Under what conditions do the mitochondria vary in size? Their diameter in the cell may change in different phases of the functional activity of the latter. Their morphology in the liver cell changes, as cited above, according to alterations of the glycogen-glucose equilibrium. Authors are agreed that they become larger and more spherical through injection of adrenalin (KATER '31, CLARK and HAIR '32, HALL and MAC KAY '33), and the change is attributed to the increase in water content of the cell (KATER '37). It is necessary to bear in mind that in these cases all the mitochondria of the cell undergo similar and simultaneous changes. It is also true that those of the neighbouring cells functioning in the same way undergo parallel changes. It must be recognized, however, that change in the functional activity of the cell is not always attended with change in diameter of the mitochondria. For example, no noticeable change in girth of mitochondria occurs in the hepatic cell of *Bufo* either during hibernation or during in-

anition (p. 169). Neither does it occur in the pancreatic cell of the mouse through different phases of its secretory activity (HIRSCH '31 a, '32).

Change in girth of the mitochondria is rather more evident in some cases during the ontogenetic history of the cell than in the preceding. It was observed by the writer that young replacing cells in the intestinal epithelium of the toad possess mitochondria which are far more slender than those of the functioning cells. The following example may serve as a further illustration. The gill-sac of the lamprey is lined with a stratified epithelium, the older cells of which are continuously replaced by younger ones. Mitochondria in the latter are slender filaments which become gradually thicker

as the cell ages (fig. 2). It is important to note that, here, all the mitochondria of a cell undergo similar and simultaneous changes in their girth, as can be seen in the figure. Change in girth of the mitochondria thus occurs, no doubt, in some cases during the ontogenetic history, i. e. during the process of differentiation or senescence of the cell, but, here also, it does not necessarily accompany the latter. It was observed, for example, that in the haematopoiesis in the yolk-sac of chick embryo, differentiation of the blood cells is not accompanied by any noticeable change in girth of the mitochondria.

Thus we have arrived at two important general rules concerning the shape and the size of the mitochondria in the cell. First, mitochondrial configuration is nearly the same in different cells of a given kind pertaining to an individual or different individuals of a given species of animal, if the cells stand both at the same stage of ontogenetic history and at the same phase of functional activity. Second, the diameter or girth of all the mitochondria in one and the same cell is astonishingly uniform, and even when change in it occurs in some cases during the ontogenetic history or the functional cycle of the cell, all the mitochondria of the cell undergo similar and simultaneous changes.

These two rules are so important that they must always be taken into consideration by students of the mitochondrial problem. Most of the studies on mitochondria must rely on fixed materials, and interpretations of data are largely influenced by the results of fixation. The above rules may serve as a criterion by which one is able to judge whether the result of fixation is faithful to nature or not. Absence of any criteria in the mind of most of the investigators has probably been the main cause which led to chaos

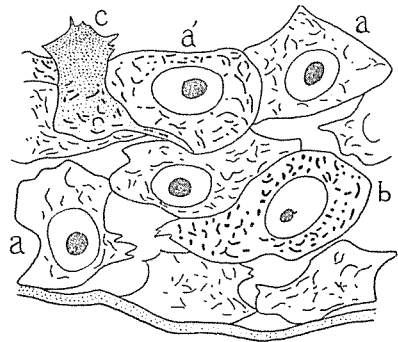


Fig. 2.—Epithelial cells of gill-sac of *Entosphenus japonicus*. a, cell at first stage; a', cell intermediate between first and second stage; b, cell at second stage; c, cell at third stage about to be desquamated. (After TAKAGI '33 a).

in the discussion of the mitochondrial problem. Mitochondria have been considered by many to be extremely variable in morphology. Though it must be acknowledged that they are semi-fluid in consistency and easily changeable in form and size, yet it is true that their changes in morphology are not without restrictions. The prevailing misbelief may be due in part to delusive results of faulty fixations and in part to the misinterpretation of the work of LEWIS and LEWIS ('15). The LEWISES observed in cultured tissues that changes in form of the mitochondria are very rapid and irregular. Any one type of mitochondria such as a granule, rod or filament may at times change into any other type or may fuse with another mitochondrion, or it may divide into two or several mitochondria. Every type of mitochondria is continually changing form and may assume as many as fifteen or twenty forms in ten minutes. It is also said that granular types may vary from minute to large spherules. Unfortunately, the LEWISES' data have been generalized and considered by many to represent the behavior of mitochondria in all living cells. It must be emphasized that such irregular and rapid changes in morphology as were reported by them are not undergone by the mitochondria in the cells *in vivo*. For example, according to the observations of GUILLIERMOND ('27), changes in form of the mitochondria in living cells of *Saprolegnia*, *Elodea* and *Iris* are not so irregular as in the LEWISES' observations. Mitochondria are carried along through the streaming movements of the protoplasm. Long filamentous types, which are mostly straight when they stand still, become wavy, zig-zag, V- or U-shaped when they are in movement. Sometimes, by the action of streaming or of an obstacle, branchings are formed on them. No remarkable changes in morphology, on the contrary, are undergone by rod-like or granular types, although they may happen to become somewhat longer or shorter. No fusion of mitochondria has been noted during extended observations and fragmentations are only rarely met with. Neither formation *de novo* nor disappearance of the mitochondria has been observed. Now, so far as changes in form of the mitochondria are restricted to such an extent, no modification of the above rules regarding their morphology may be necessary. As there are only a few animal tissues which permit extended observations of the mitochondria in living condition, it was but natural that the LEWISES' observations received high appreciation. It must be recognized, however, that cells *in vitro* are not under entirely normal conditions, but are liable to physicochemical changes through imbibition of the cytoplasm, which may be the cause of irregular and rapid changes in form of the mitochondria. According to the observation of HIRSCH ('32) on the living pancreatic cells of the mouse, on the contrary, shifting in position and changes in form of the mitochondria during the secretory phase of the cell are but insignificant. Studies on the mitochondria of the cell *in vitro* are valuable by themselves, but no direct information can be expected therefrom on those of the cell *in vivo*. CHAMBERS ('15) stated that in the surviving spermatogonial cells of the grasshopper mitochondria appear to be



constantly shifting in position and to be disappearing and reappearing in the cytoplasm. In close association with the work of the LEWISES, this was widely taken for an evidence of their labile existence. But there remains ample room for doubt whether they really disappeared and reappeared in the cytoplasm. It is an open question whether they did not disappear from and reappear into the focus of the microscope by their constant shifting in position. Disappearance of mitochondria in the cytoplasm is known to be usually preceded by a series of degenerative changes. It is hardly conceivable that it was in his case alone so simple and instantaneous a matter as he described it.

Mitochondrial configuration in a given kind of cells is definite for a given stage of ontogenetic history and a given phase of functional activity of the latter. What factors are here involved is an interesting question. Inasmuch as it is much alike in similar tissues of nearly allied animals, it may safely be said with COWDRY ('18) to be a property ingrained during the phylogeny in the very organization of the cell. But by what factors it is determined physicochemically is a question that cannot be answered to-day, although water content of the cytoplasm may be an important element. The present writer is convinced that there is rich reward waiting for the students of protoplasm if they direct their effort toward elucidation of this problem.

### 3. On the localization and arrangement of the mitochondria in the cell.

Mitochondria are present in the cytoplasm, but not, of course, in the nucleus. They are not present, however, everywhere in the cytoplasm. In amoebae they are contained numerously in the endoplasm, but not in the ectoplasm (p. 172, fig. 5). Here should be recalled the generally held opinion that the ectoplasm differs in consistency from the endoplasm. The former represents a "plasmagel," and the latter a "plasmadol" (MAST). Or, it is also said that between the hyaline zone of ectoplasm which is usually liquid and the fluid granular endoplasm there is another zone of granular ectoplasm which is relatively solid (CHAMBERS). Further evidence is available to show that the localization of mitochondria in the cell is conditioned by the colloidal state of protoplasm. All the authors agree that mitochondria are absent in dividing cells within the interior of the mitotic spindle (p. 171, TORRACE, TERNI, LEWIS and LEWIS, LEVI etc.). The mitotic spindle is known to be far greater in consistency than the surrounding cytoplasm and to be in a gel state (MORGAN, SHEARER, CHAMBERS etc.). Furthermore, it is well known and observed also by the writer that mitochondria are not in direct contact with the centriole. A survey of literature indicates that the distance between the two corresponds in all probability with the bulk of the centrosphere. According to CHAMBERS, the centrosphere itself is fluid in consistency, but its outer wall is very viscid, representing at the same time the most gelled portion of the aster. Thus, the absence of mitochondria within the centrosphere seems to be conditioned also by the colloidal state of the protoplasm. In this connection it may be of interest to remind ourselves of a

fact, which is a common observation of students of mitochondria, but to which no attention has ever been paid: mitochondria are never in direct contact with the outer wall of the cell, there being always some distance between the end or side of the outermost representatives of the former and the latter. Yet whether this also is due to the difference in consistency between the cortex and the interior of the cell is an open question, inasmuch as the presence of a more or less solid cortex of appreciable thickness such as occurs in many Protozoa and some egg cells has not yet been determined in somatic cells of the Metazoa.

Next, we turn to the problem of the orientation of mitochondria in the cell. It is well known to cytologists that, in typical gland cells of vertebrates, filamentous mitochondria are directed from the base toward the free end of the cell. This mode of orientation of the mitochondria is not unique to gland cells, but also characterizes cells of gland-like function such as the

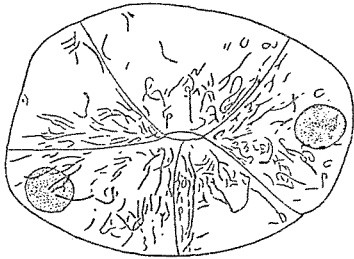


Fig. 3.—Orientation of mitochondria in hepatic cells of *Bufo vulgaris*. (After TAKAGI '29 a).

epithelial cells of urinary tubules of the kidney and those of the plexus chorioideus. It is also found in the hepatic cells of the anurans (fig. 3) (the writer in *Bufo*, OKAMOTO '24 in *Rana*). POLLISTER ('32 a) observed also such an orientation of filamentous mitochondria in the hepatic cells of *Amphiuma*, which he suggested might be due to the diffusion current through the cell body from the blood vessels toward the lumen. According to the observations of HIRSCH ('31 a, b) on the living pancreatic cells of the mouse, of which the preëxist-

ing mitochondria have been made to disintegrate by the action of RÖNTGEN rays, a new filamentous mitochondrion first appears at the basal region of the cell with its length running almost parallel with the base. It begins to move and continues to migrate toward the free end of the cell, elongating gradually and often dividing into two, to become oriented almost parallel with the cell axis. He made no explanation of this migration, which probably cannot be attributed to anything other than the diffusion or water current in the cell protoplasm.

The columnar epithelial cells of the intestine are provided with two more or less condensed groups of filamentous mitochondria, one just beneath the cuticular border and the other at the base of the cell. Orientation of the mitochondria parallel with the long axis of the cell is more pronounced in the former (fig. 4). CHAMPY ('11) is of the opinion that the epithelial cells of the intestine are both secretory and absorptive in function, the particular arrangement of the mitochondria in the cell indicating the existence of double polarization in two directions. But no evidence is as yet available which shows that the mitochondria beneath the cuticular border are associated with secretory activity. On the contrary, there is some

evidence in favour of the view that they subserve, together with those at the cell base, the absorptive rôle of the cell. From the fact that the mitochondria and GOLGI material in the columnar epithelial cells of the ciliary body of the eye closely resemble those in the intestinal epithelium in their arrangement, MA and PILLAT ('29) thought of an absorptive activity on the part of the former, which they could prove experimentally by injecting cod-liver oil etc. into the posterior chamber and by demonstrating these afterwards in the epithelial cells. Fat droplets were seen to be attached on the surface of the mitochondria.<sup>1)</sup> The filamentous mitochondria which are oriented here parallel with the long axis of the cell appear to play a rôle of determining the loci of migration of the fat droplets. Although both MUKAI ('29) and IWA-SAWA ('30) are of the opinion that the ciliary body may be secretory in function, the results of their investigation are far from convincing. The orientation of the mitochondria in the columnar epithelial cells of the intestine as well as in those of the ciliary body may probably be best explained by diffusion or water current which flows in this case, opposite in direction to that in a gland cell, from the free toward the basal end of the cell.

Presence of the aster often plays a particular part in determining the arrangement of mitochondria in the cell. Radial orientation of mitochondria around the centrosome is known to occur in different kinds of cells, namely in the fibroblasts (W. H. LEWIS '19), plasma cells (WALLGREN '11), leucocytes (POLLISTER '32 b), blood cells of some invertebrates (VOLKONSKY '33), as well as in spermatogonial cells (GAMBIER '29). Although the last named author considers with CHAMPY ('13) that the radial arrangement of mitochondria is due to an expelling action of the centrosome, this is not a satisfactory explanation. It is known in the fertilization of echinoderm eggs that when the aster accompanying the male pronucleus enlarges and the centrosphere grows in size, a centripetal flow of fluid toward the centrosome is so intense that not only the female pronucleus, but even oil drops move toward the centre of the aster (GRAY). Here the centrosome may be said to exert an attractive rather than an expelling action. The latter may, however, actually take place when the centrosphere is at the phase of diminishing in size. At any rate, it cannot be questioned that the radial arrangement of mito-

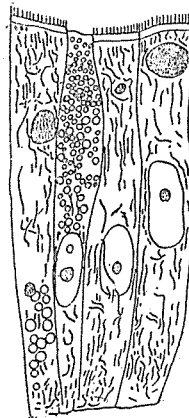


Fig. 4.—Columnar epithelial cells and a gland cell of mid-intestine of *Entosphenus japonicus*. Besides mitochondria, debris of worn-out erythrocytes and fat droplets are indicated in the former and secretion granules in the latter. (After TAKAGI '33 a).

1) The figure given in their report cannot be interpreted otherwise, although they described the fat droplets as embedded in the filamentous mitochondria.

chondria around the centrosome is due to the centripetal or centrifugal flow of protoplasm toward or away from the latter. MAXIMOW ('13) states that filamentous and rod-shaped mitochondria in living plant cells migrate under the influence of the protoplasmic streaming with their length directed parallel with the latter. In the case of mitochondria around the centrosome, it may be that they are arranged radially by the centripetal or centrifugal flow of hyaloplasm and maintained in their orientation as long as the astral rays continue to exist. KINGSBURY ('12) supposed that, being reducing substances, mitochondria carry a positive electrical charge and accumulate around the centrosome in order to deliver it. Although their mere assemblage around the centrosome may be explained by his hypothesis, it does not suffice to elucidate the radial arrangement, extending sometimes almost to the outer wall of the cell, of elongated types of mitochondria.

Thus we are in a position to say that the diffusion or water current in the cell is responsible for the regular orientation of mitochondria characteristic of gland cells and the like as well as of cells of absorptive function, and that the centripetal or centrifugal flow of protoplasm toward or away from the centrosome is an agent for determining the radial orientation of mitochondria around the latter.<sup>1)</sup>

It is a conspicuous fact that filamentous mitochondria in the cross-striated muscle fibres of amphibians, fishes and cyclostomes are oriented parallel with the bundles of myofibrils. Obviously their arrangement is a forced one, for being elongated forms, they cannot be oriented otherwise in a narrow, limited mass of sarcoplasm between bundles of myofibrils. This may clearly be understood from the fact that in a relatively broad mass of sarcoplasm beneath the sarcolemma they are arranged quite at random. Incidentally it may be remarked, but requires no further explanation, that the very presence of formed materials in the protoplasm, such as the GOLGI material, secretion granules, fat droplets, glycogen etc., may act in some way and to some extent in modifying the localization and arrangement of mitochondria in the cell.

HORNING ('27) states that the arrangement of the mitochondria in the surface layer of the ciliates, *Nyctotherus* and *Paramoecium*, is strikingly regular, though the two differ in mode. Elongated forms of mitochondria in the surface layer in the middle region of the body are arranged, in *Nyctotherus*, side by side and at right angles to the longer axis of the cell, whereas, in *Paramoecium*, they are arranged end to end and parallel with that axis. This difference is due according to him to the difference in width of intervals between the myonemes, which is larger in the former than in the latter. Interesting as his findings may seem at first sight, it must, however, be recognized that those granules between the myonemes which

---

1) It may probably be asked whether or not the micellar submicroscopic structure of the cytoplasm participates in a way or another in determining and maintaining the orientation of mitochondria in the cell, but it is wholly impossible in the present state of our knowledge to answer the question.

are here dealt with are not true mitochondria. According to the recent investigation of the present writer, which has not yet been published, the granules situated between the myomeres of the ciliates must strictly be distinguished from the true mitochondria embedded in their internal protoplasm. The granules in question are found to be different from the latter in the following points. (1) They are, in *Spirostomum ambiguum* and *Colpidium colpoda*, more resistant to acetic acid than the mitochondria. (2) They retain, in the above species and others, iron hematoxylin stain more intensely than the mitochondria. (3) They are stained, in *Blepharisma lateritium*, by a natural pigment, zoopurpurin, but the mitochondria are not. (4) In a certain heterotrich ciliate, they could be stained brilliantly by vital staining with neutral red, but the mitochondria could not. LÉGER and DUBOSCQ ('16) report that the mitochondrial filaments in *Balantidium* are arranged in the pharyngeal region and its neighbourhood perpendicular to the body surface, in the middle region of the body for the most part parallel with the longer axis of the cell, and in the posterior region again perpendicular to the body surface. HALL ('29) also described a spiral arrangement of mitochondria in the peripheral region of the flagellate *Peranema*. From the foregoing, however, the question may be raised whether these authors described true mitochondria or not.

To summarize briefly what has been concluded in the present section :

The localization of mitochondria in the cell seems to be conditioned by the colloidal state of the protoplasm. Their particular arrangement and orientation around the centrosome and in some types of cells may probably be attributable to the presence in the cell of protoplasmic streaming, diffusion or water current. It is obvious, however, that the presence of other formed materials in the protoplasm may act in some way and to some extent in modifying the localization and arrangement of the mitochondria in the cell.

4. On the quantity and changes in quantity of the mitochondria of the cell.

During many years of study attention has always been paid by the present writer to what kind of cells are rich and what kind of cells poor in mitochondria. The first suggestion of value came to him from his study of various kinds of amoebae. It was found that the larger types of amoeba are always furnished with more abundant mitochondria than the smaller (fig. 5). It

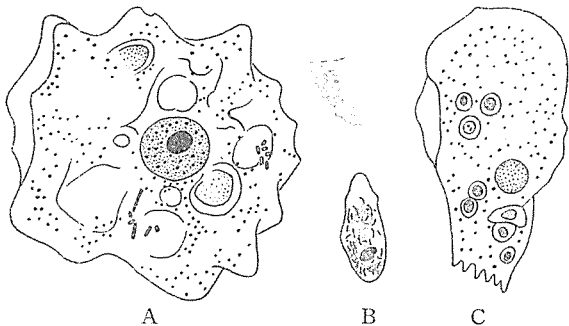


Fig. 5.—Three different types of amoebae. Mitochondria are granular in A and C, and shortly filamentous in B.  $\times 800$ . (After TARAGI '34 b).

seems, then, that the quantity of mitochondria of a cell is dependent on the quantity of protoplasm. Much other evidence is available among his own data, showing that tissues or cells which are rich in protoplasm are also rich in mitochondria. Thus it was found that in the toad the cardiac muscle contains more mitochondria than the skeletal muscle and of the constituent muscle fibres of the latter, the red is provided with more mitochondria than the pale. The PURKINJE cells of the conducting system of the heart, which are known to be rich in protoplasm, were found to possess abundant mitochondria (p. 170). The specialized, darkly coloured muscles of the fishes, commonly called in Japanese "Tiai," consist of muscle fibres poor in myofibrils and rich in sarcoplasm. They were revealed to be especially rich in mitochondria (unpublished). It is also known from a survey of literature that mitochondria are abundant in cells with a large mass of protoplasm, such as the megakaryocyte (JORDAN '14, UETANI '38) and the osteoclast (CHANG '31), and are scanty in cells with only a small mass of protoplasm, such as the lymphocyte, thrombocyte and blood platelet (COWDRY '14, see also fig. 6). Much more evidence may be cited in favour of this view, but none trustworthy against it.

On the other hand changes, if any occur, in quantity of the mitochondria accompanying functional activities of the cell are not so significant as have generally been supposed. The view that mitochondria in gland cells are directly transformed into secretion granules was received by many workers as well as by most of the biologists as the most plausible theory on the origin of the latter. But the lack of convincing proofs and considerations on theoretical grounds hindered some investigators from accepting the hypothesis (see also p. 196). The experiment made by HIRSCH ('32) on the living pancreas of the mouse may be regarded as crucial. He exposed a thin layer of the pancreatic tissue under intact circulation outside of the body and observed it for long hours under high magnification of the microscope. In the pancreatic cells made practically free of preëxisting secretion granules by injecting pilocarpine, new granules make their first appearance at the surface of mitochondria situated near the base of the cell, become detached and migrate, growing gradually in size, toward the free end of the cell. Remarkable is the fact that the mitochondria do not undergo any changes during the process, neither disintegration nor even loss of substance taking place.<sup>1)</sup> Turning to the process of absorption, it was contended by KITAMURA ('28) that mitochondria in the columnar epithelial cells of the intestine become, especially during fat absorption, disintegrated and transformed during the process. But practically no changes could be observed there by CRAMER and LUDFORD ('25), who expressed the opinion that mitochondria do not take an active part in the process of fat absorption and

---

1) RIES ('35), who could not agree with HIRSCH on the origin of secretion granules, ascribed it to the lipochondria which are permanent cell structures and are independent of the mitochondria.

assimilation. According to the observation of MA and PILLAT ('29) on the columnar epithelial cells of the ciliary body of the eye, fat droplets, which are absorbed and appear in the cells after cod-liver oil has been injected into the posterior chamber, become temporarily attached to the surface of mitochondria, but no disintegration of the latter takes place. In view of these results, it seems difficult for the writer to acknowledge at once the result of KITAMURA as well as those of TIRELLI ('28), LIU ('30) and SAITŌ ('33) to the effect that mitochondria disintegrate in functioning intestinal epithelia. Further experimentation to settle this point is to be hoped for. At present, however, the writer is of the opinion that changes, particularly in quantity, of the mitochondria accompanying functional activities of the cell are not very significant. In this connection it must be recalled as a further evidence that the mitochondrial content of the cells of the gastric gland, the intestine, the muscle etc. of the toad during hibernation, in which the functional activities of the organs are at an extremely low level for several months, was found not to differ so much as had been expected from that during the summer (p. 169).

A large volume of literature has been accumulated on mitochondrial changes under various abnormal conditions, such as vitamin deficiency, inanition, infections or poisonings, and by various agents, such as RÖNTGEN or radium rays, hyper- or hypotonic solutions, acids or bases, or alterations of temperature. Three modes of reactions on the part of the mitochondria are recognized—qualitative, quantitative, and topographical, which may occur singly or in combination. As to the quantitative changes which are now in question, mitochondria are reported in many cases to decrease in number or to disappear entirely. Cases are comparatively rare in which they are shown to increase in number. Disintegration or dissolution of the mitochondria is usually preceded by a series of qualitative changes including alterations in form, size and stainability. It may be remarked incidentally that it is a conspicuous fact that the qualitative changes of mitochondria as the response to diversified cellular injuries are generally alike. It probably shows that the latter do not act directly upon the mitochondria, but after a particular series of reactions give rise to some physicochemical change of the protoplasm which is the immediate cause of the results observed. Thus, NISIO ('31, '32) arrived at essentially the same results, including changes in contour, loss in stainability and ultimate disintegration of the mitochondria, after exposing living pancreatic cells of the mouse to RÖNTGEN or radium rays, to hyper- or hypotonic saline solutions, or to unfavorable concentrations of hydrogen ions, although he treated the subjects only separately and did not imagine possible existence of a common cause that may underlie all his results. Now it is curious that in spite of the immense volume of literature no effort has ever been made to find out to what extent a living cell can endure degenerative changes of its mitochondrial garniture. A first approach to this problem, it may be said, was made by HIRSCH ('31 b) in his work on the analysis of restitution of the secretion material in the

mouse pancreas by means of RÖNTGEN rays, in which it was shown that mitochondria can be formed *de novo* in the cytoplasm after all the pre-existing ones have been destroyed to such an extent as to lose their normal smooth outline, and that the cell awakes in its rôle of generating secretion granules first when the amount of newly formed mitochondria nearly reaches that of the original ones. If the result of this investigation be allowed to be generalized, it may be said that the cell can not perform its normal activity in a condition in which the mitochondria are greatly decreased or absent.

The present writer is of the opinion that, in normal living cells, marked changes in quantity of the mitochondria can occur only during the ontogenetic history, i. e. during the process of differentiation or senescence, of the cell. This is clearly indicated in embryological studies. Prevailing is the opinion that mitochondria are rich in embryological cells and relatively

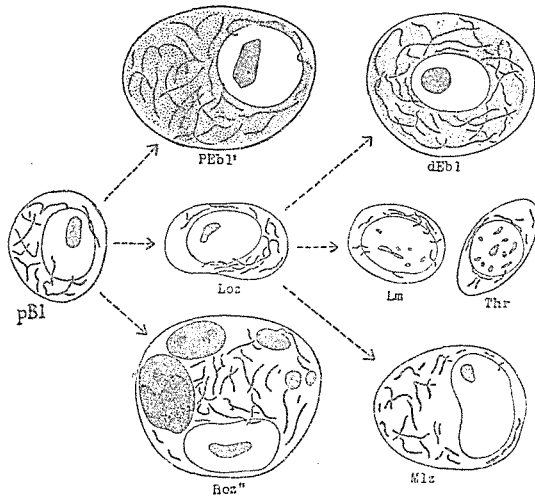


Fig. 6.—Differentiation of blood cells and mitochondria. pBl, primitive blood cell; pEb', primitive erythroblast with haemoglobin; Loz, lymphoid cell; Hoz'', histioid cell with debris of erythrocytes; dEb1, definitive erythroblast with haemoglobin; Lm, lymphocyte; Thr, thrombocyte; Mlz, younger stage of myelocyte.  $\times 1600$ . (After TAKAGI '31).

poor in matured cells. But in reality, there do exist two different modes of cellular differentiation, one accompanied with increase and the other with decrease of the mitochondria. This is clearly shown in the case of my study on the haematopoiesis in the yolk-sac of chick embryo (p. 171). It may easily be seen from fig. 6 that the differentiation either from the primitive blood cells of the primitive erythroblasts and the histioid cells or from the lymphoid cells of the definitive erythroblasts and younger stages of the myelocytes is accompanied with increase, and that from lymphoid cells of the lympho-

cytes and the thrombocytes is accompanied with decrease to a greater or less degree of the mitochondria. Furthermore, this study together with considerations from other lines led me to believe that, in normal cells, changes in quantity of the mitochondria are dependent on the quantitative changes of the cytoplasm. Increase or decrease of the former is attended with corresponding increase or decrease of the latter. In individual cases, this relation has already been noticed by several authors. That increase in the amount



of the mitochondria is accompanied by increase in that of the cytoplasm was observed in cells of the regenerating tissues (ROMEIS '13), in the formation of osteoclasts (CHANG '31), in the oogenesis of *Cambarus* (KATER '28), and also in the degeneration of muscular tissue (DEJKUN '29). GOETSCH ('16) reported in a case of "foetal adenoma" of the thyroid gland that the mitochondria are strikingly abundant in the parenchymal cells, which are generally tall columnar and contain relatively abundant cytoplasm. In the development of the anterior pituitary of the domestic fowl, it was found by KATŌ and NISIDA ('35) that, during the period from 30 to 90 days after hatching, mitochondria increase in number in the  $\beta$ -cells in accompaniment with the growth of cell body; that in hens 4 or 5 years old they are greatly diminished in both the  $\alpha$ - and  $\beta$ -cells which have also become smaller in size than in the prime, and further that, in castrated animals, they are increased in the  $\beta$ -cells which are hypertrophied twice as large as in the control. But there are not a few authors who speak only of quantitative changes of mitochondria, leaving out of consideration changes in size of the cell, in whose papers, however, the latter changes are clearly indicated in figures. Such is the case for example, when CRAMER and LUDFORD ('26) state the quantitative changes of mitochondria in the rat's thyroid gland, and also when NICHOLSON ('23) reports the decrease of mitochondria accompanying senescence of the thyroid gland cells of the guinea pig.

Through years of studies on a variety of tissues, the present writer has been led to believe that cells pertaining to a definite type and having a nearly definite size possess mitochondria to much the same quantity, and that, in those cells in which the mitochondria are uniformly distributed in the cytoplasm, the density of the former in relation to the latter is nearly definite according to the type of cell. In fact, about the nerve cells the latter half of this statement had already been shown to be the case by quantitative studies. THURLOW ('16, '17) counted the number of mitochondria contained in a definite volume of cytoplasm of different types of nerve cells of the mouse and found it to be practically the same in different cells of the same type; moreover, no differences were noticed according to the individual. A similar study was made by RASMUSSEN ('20) about the nerve cells of the central nervous system and the spinal ganglion of the woodchuck. As a result, it was found that the number of mitochondria contained in unit volume of cytoplasm is definite according to the type of cell and is modified neither by profound dormancy during hibernation nor by prolonged in-anition. This affords strong evidence in support of the above expressed opinion of the writer that the mitochondrial content of the cell is not liable to quantitative changes of any marked degree in different levels of the functional activity of the cell. It has also been shown in the preceding pages that noticeable changes in quantity of the mitochondria do occur sometimes accompanying the process of cellular differentiation or senescence. Here, however, as they are attended with simultaneous and corresponding changes in volume of the cytoplasm, no marked and abrupt changes may probably

be expected in the number of mitochondria contained in unit volume of the cytoplasm. Hence it may be supposed that the latter may not be very different in magnitude even about cells belonging to different categories of the same animal, excepting those cells which possess long filamentous mitochondria. This theoretical deduction lacks as yet any proof of its own, but presents a subject of great importance in the biology of mitochondria. It is clear that in investigations of this sort allowances should be made for some cytoplasmic differentiations or products of metabolism which largely influence relative volume of the cytoplasm in relation to the number of mitochondria. The present writer obtained evidence which tends to suggest, a step further, that the number of mitochondria contained in unit volume of the cytoplasm may not be very different even among cells or protoplasts far removed from one another in the evolutionary scale. The number of mitochondria contained in 660 cubic microns of the cytoplasm was found to be approximately equal in the two species of ciliates, *Spirostomum ambiguum* and *Blepharisma lateritium*, corresponding to the number of 121 million per cubic mm. of the cytoplasm (p. 175). This is in the same order of magnitude with those obtained by THURLOW and by RASMUSSEN about the various types of nerve cells, i. e. 178-284 million and 186-354 million respectively per cubic mm. of the cytoplasm. Surely it is hazardous to conclude from this alone that the number of mitochondria contained in unit volume of cytoplasm is nearly alike in varied types of animal protoplasm. It may be safe in the present state of our knowledge to confine ourselves to the statement that the number of mitochondria per unit volume of cytoplasm is much the same among different cells of the same type and, seemingly, among protoplasts of very near affinity.

COWDRY and COVELL ('27) introduced the term "mitochondria-cytoplasmic ratio" by which was meant the ratio the total sum of the surface of mitochondria bears to the volume of the cell multiplied by 100. It may be inferred from the above lines that the ratio will remain practically the same among cells of the same type containing mitochondria in approximately equal numbers per unit volume of the cytoplasm, when the cytoplasmic constituents are in the form of granules or rods and are of approximately the same size. Under these conditions the estimation of the mitochondria-cytoplasmic ratio may be a comparatively easy matter. Some difficulties are presented when mitochondria appear in filamentous forms, which, however, may be overcome by taking the trouble, as COWDRY and COVELL did, of measuring the length of individual filaments. In the kidney of the albino rats, these authors actually found that the ratio is definite according to the type of cell and is greatest in the proximal convoluted tubule (192.62), practically identical in the distal convoluted tubule (144.44) and ascending limb of HENLE's loop (138.14), and least in the descending limb of HENLE's loop (60.11). They suggested that the highest value in the proximal convoluted tubule might be related to the fact that the function of the portion is radically different from that of other sections. But whether such a dif-

ference in the ratio, relatively small, may safely be attributable to the difference in function seems to be still an open question, until additional accumulation of data along this line of research leads us to admit such a conclusion.

To sum up the conclusions of the present section:

The mitochondrial content of normal cells does not undergo quantitative changes to any marked degree during the functional cycle of the cells. On the contrary, it evidently increases or decreases in some cases during the process of cellular differentiation or senescence, being accompanied here, however, with simultaneous increase or decrease respectively in quantity of the cytoplasm. The number of mitochondria per unit volume of cytoplasm is much the same among different cells of the same type.

#### **IV. On the nature and functional significance of the mitochondria**

In the preceding chapter it was concluded that mitochondria are present in the cytoplasm of all animal cells except some of those which are at the terminal stages of their ontogenetic history, that there exist some general rules that govern their form, size and arrangement in the cell. Further it was shown that the mitochondrial content of a cell is not liable to quantitative changes to any marked degree during the functional cycle of the cell, but depends on the quantity of the cytoplasm, either increase or decrease of the former being attended with increase or decrease respectively of the latter, and that the number of mitochondria per unit volume of cytoplasm remains much the same among different cells of the same type.

The present chaotic state of the mitochondrial problem may perhaps be attributed to the fact that most of the investigators put off their first duty of finding out rules or laws to which morphological features of the cytoplasmic constituents in question are subjected, and directed their efforts almost exclusively to the search for the nature and functional significance of the mitochondria. No natural phenomena, however complicated they may appear at first sight, are without some rules or laws. It can hardly be conceived that the mitochondria which are universal constituents of the living substance do not obey some definite rules. In reality, as we have seen, there do exist some rules which govern their form, size, arrangement in the cell and their quantitative aspects. The present writer believes that further evidence confirmatory of these rules will, in future days, be repeatedly brought forward by investigators working with ample technical precautions. Any arguments regarding the nature or the functional significance of the mitochondria must be based upon, or at least in harmony with these rules.

The idea that mitochondria are chiefly of phospholipin nature with perhaps a small amount of protein in combination, has prevailed for decades and has often been utilized for theoretical considerations on their functional significance with respect to diverse intracellular phenomena, although, lacking direct proofs, it is largely based on circumstantial evidence as was

summarized by COWDRY ('18, '24). Recently, BENSLEY and GERSCH ('33) made a thorough criticism of the literature on the chemical composition of mitochondria, pointed out the inappropriateness of applying microchemical tests to intact cells, and inaugurated a study with so-called undenatured materials. Liver tissues of *Amblystoma* were frozen in liquid air immediately on removal from the body, completely dried and embedded in vacuum, and cut into sections. No modification in shape or stainability of the mitochondria resulted through extraction with acetic acid, alkalis, alcohol, chloroform, acetone, sulphuric ether, or petroleum ether. The mitochondrial substance, further, did not melt at the temperature of 48-50°C, as had been expected from data on surviving cells. The conclusion was drawn that its main mass is protein in nature as was indicated by the positive result with MILLON'S reaction and by the complete digestion with artificial gastric juice. BENSLEY and HOERR ('34) ground or crushed guinea-pig's liver in a mortar, separated mitochondria from impurities by repeated centrifugation, and subjected the mitochondrial substance so obtained to direct chemical analysis. 43.6% of the whole belonged to fatty substance, which, however, was shown not to contain any lipoid as had previously been supposed. In addition, two proteins with different isoelectric points were also detected. As was acknowledged by the authors themselves, a little loss of substance may probably have occurred during the process.

A remarkable opinion was expressed by GIROUD ('29) to the effect that the mitochondria-hyaloplasm as a whole represents a system consisting of two kinds of phases, which are qualitatively of the same nature, and differ only quantitatively. No particular substances, which do not exist in the hyaloplasm, are contained in the mitochondria. This view is in harmony with the observation of HIRSCH ('31 a, b) that mitochondria may be formed *de novo* in the cytoplasm, after the preëxisting ones have been disintegrated by the action of RÖNTGEN rays, and grow rapidly in length with subsequent binary fission. Theories, which contend either that the mitochondria can only arise from preëxisting ones (GUILLIERMOND '18) or that they have their origin from the nucleus (SAGUCHI, HOSSELET, RADU and others), must now be abandoned.<sup>1)</sup> Our view that the mitochondrial content of a cell increases or decreases in accordance with a similar change in amount of the cytoplasm may probably be best interpreted by the hypothesis that the mitochondria represent only different phases of the hyaloplasm and the whole system, i. e. the cytoplasm, comes to an equilibrium when the total sum of volumes or surfaces of the former stands at a certain numerical ratio to the volume of the latter. It may be of interest here to call to mind the experiment of GOSS ('30). It was observed that through dissection with microneedles, specific granules of the neutrophile leucocytes were separated

---

1) It was also shown by MILOVIDOV ('33) that the mitochondria of the vegetative cells give a negative nucleal reaction and therefore do not contain any thymonucleic acid which is the most characteristic element of the cell nucleus.

from the cytoplasmic matrix and were seen in Brownian movement both inside and outside the cell, whereas the mitochondria either of those cells or of the lymphocytes could not be released from their combination with the matrix in this way. This probably shows that the mitochondria, which are not very different in chemical nature from the hyaloplasm, are also physically more intimately connected with the latter than with any other granules. That mitochondria are gels in the hyaloplasm which is usually in sol state may be understood from the microdissection experiments of CHAMBERS ('15, '24) as well as from their attitude toward obstacles in protoplasmic streaming (MAXIMOW '13, GUILLIERMOND '27). Thus the cytoplasm of a cell may probably be interpreted to consist of a number of dispersed gel phases, the mitochondria, suspended in a continuous sol phase, the hyaloplasm, both kinds of phases being, however, composed qualitatively of similar sorts of substances.

To ALTMANN ('90) is due the credit of first systematically describing mitochondria in animal cells, but his theory on their nature met severe criticism. He regarded them together with the bacteria as elementary organisms or morphological units of the living substance, the only difference being that the mitochondria can live only inside the cell, whereas the bacteria are capable of independent existence. His opinion that the hyaline cytoplasm serves only as the ground substance embedding the living units and is in itself insignificant for vital processes, is wholly incompatible with modern conceptions of cellular physiology. Yet this mistake of regarding the mitochondria as microorganisms was followed by several investigators. Among others, WALLIN ('23 etc.), cultivating liver tissues of new-born rabbits *in vitro*, reported that the hepatic cell mitochondria migrated from the cell and proliferated in the culture medium and he considered that the mitochondria are bacterial organisms symbiotically combined with the tissues of higher organisms. In his experiment of cultivating adipose tissues of the sex organs, PORTIER ('18) obtained also multiplication of bacterial organisms, which he declared to have been derived from the mitochondria. Doubt always arises regarding experiments of this sort as to whether the mitochondrial colony as it is called by the investigators, really represents multiplication of mitochondria outside the cell, or is not rather a colony of true bacterial organisms inoculating the culture. The bacterial theory was severely criticized by several investigators (COWDRY and OLITSKY '22; COWDRY '23; MILOVIDOV '28 a, b etc.), who pointed out or indicated experimentally, differences that exist between mitochondria and true bacteria. Among other objections, however, probably the most potent is the finding made by HIRSCH ('31) that the mitochondria are capable of forming *de novo* in the cytoplasm of living pancreatic cells of the mouse.

MEVES in 1908 propounded a general theory according to which all the visible differentiations which develop in different types of cells during ontogenesis were regarded as modifications of the mitochondria. These were thought to be concerned in the formation, for example, of myofibrils, neuro-

fibrils, secretion granules, fat droplets and the like (MEVES '07 a, b, '09; DUESBERG '09; HOVEN '10, '11; ARNOLD '12 a, b etc.). This apparently plausible hypothesis which contends that the visible differentiations are derived from the cytoplasmic constituents which are also visible and of nearly the same dimension, and especially the view that the mitochondria in gland cells are directly transformed into secretion granules has long prevailed and found many supporters (e. g. SAGUCHI '20, MORELLE '25, TSUKAGUCHI '26, MITAMURA '34). But not only was the observational evidence of the mitochondrial origin of most of the visible differentiation products of the cell brought into serious doubt by later investigators (LEVI '16; GURWITSCH '13; LEWIS '17; LAGUESSE '26 a, b; GUILLIERMOND '27; ALEXENKO '32 etc.), but COWDRY ('18, '24) also suggested the theoretical improbability that the mitochondria which are not very different physically and chemically in different cell categories, could be transformed into so diversified products of differentiation. That the secretion granules in gland cells are not direct derivatives of the mitochondria seems to have been definitely proved by the works of HIRSCH ('32) and RIES ('35) (see also p. 188). In the writer's own studies in which glandular tissues, such as the fundic gland, the pancreas, the liver or the kidney of *Bufo* (p. 169) or the luminous organ of *Cypridina* (p. 174), were used as the material, he searched eagerly and constantly for any figures indicating direct transformation of mitochondria into secretion granules, but nothing of the kind could be obtained. Only in the light cells of *Luciola*, mitochondria were sometimes seen to be somewhat thickened at the ends or at the intermediate portion. These thickenings were thought to be due probably to the deformation of the mitochondrial substance by fixation, or to represent initial stages of developing photogenic granules united with the substance of the mitochondria through the action of the fixative. In the PURKINJE cells of the conducting system of the heart, also, no evidence was obtained in favour of the view that myofibrils are direct derivatives of the mitochondria (p. 170). In the haematopoiesis in the yolk-sac of chick embryo, further, no indications were available to show either that the mitochondria in the erythroblasts are forerunners of haemoglobin or that those in the myeloblasts give rise to leucocytic granules. Thus, we are unable to support the dictum of MEVES and his followers that the mitochondria in the animal cells are freely transformed into cytoplasmic differentiations of this sort or any other. It must probably be acknowledged, however, that some of the mitochondria in the plant cells are directly transformed into plastids in view of the fact that the two kinds of granules resemble each other both physically and chemically and that the transformation is actually observed in living plant cells (MAXIMOW '13, GUILLIERMOND '32, '34).

The hypothesis that the mitochondria mediate protoplasmic respiration seems to have been founded on the fact that their occurrence is universal in the living cells. As stated in the foregoing pages, they are not only present in all the animal cells except some which are at the terminal stages of ontogenetic history, but also they are, in many cases, almost uniformly

distributed in the cytoplasm, their number per unit volume of the cytoplasm, moreover, remaining fairly constant among cells of the same type. Their importance as a universal constituent of the living protoplasm can not too much be emphasized, but we have no direct evidence for their supposed rôle in cellular respiration. The suggestion of KINGSBURY ('12) and that of MAYER, RATHERY and SCHAEFFER ('14) that the mitochondria participate in oxidations and reductions in the protoplasm is based merely on indirect evidence and on the not well-established knowledge of their chemical composition. More recently, JOYET-LAVERGNE ('28, '29) showed that mitochondria are coloured purple by sodium nitroprussiate and thought them the seat of glutathione which is concerned in the oxidations and reductions in the cell. It must be recognized, however, that the reaction given by sodium nitroprussiate is by no means specific to glutathione, but is characteristic of -SH groups in general. In his paper on the microchemical studies on the mitochondria in the intestinal epithelium of *Ascaris*, GIROUD ('29) rightly concluded that the positive reaction obtained is due to the presence of some -SH compound in their chemical composition. According to his later work ('31) on various sorts of animal tissues, the substance in the mitochondria giving positive reaction against the reagent is not glutathione itself, which permeates probably through the entire protoplasm, but some water-insoluble -SH compound, which is present not only there, but in the myofibrils and the hyaloplasm as well; and although the reaction is conspicuous at the formed constituents of protoplasm such as the myofibrils and the mitochondria, its intensity depends merely on the concentration of the substance. Thus, if the supposition by analogy is allowed that the substance mediates oxidations and reductions in some way, the rôle played by the mitochondria in cellular respiration may be but subordinate. As was pointed out by NEEDHAM and NEEDHAM ('27), the well-known fact that Janus green B, the rH of which lies at about 5.2 (pH 7.0), is often reduced to diethylsafranine on the surface of the mitochondria might mean that the oxidation-reduction potential of these bodies is at a very low level. But theoretically, this does not necessarily signify that they are cytoplasmic locations where active reductions actually take place. A similar interpretation may probably be applicable to the findings of JOYET-LAVERGNE (1928 etc.) that pyrogalllic acid, metol, hydrochinone, diamidophenol etc. are oxidized, and gold chloride and silver nitrate reduced on the mitochondria of some plant and animal cells, that leucobases of methylene blue, Nile blue and toluidin blue are oxidized on those of some Sporozoa and Fungi, and further that cobaltous salts are oxidized on the surface of mitochondria of the *Triton* erythrocytes. To what extent, in living cells, oxidation-reduction phenomena are going on on the surface or in the interior of the mitochondria is at present quite obscure. But the possibility cannot be denied that some oxidizing enzymes are more concentrated on the surface of mitochondria than elsewhere in the cytoplasm, or that some sort of substances are adsorbed and concentrated to become oxidized or reduced on their surface in

the sense alluded to in the surface film theory (see below). At any rate, it does not seem justified to-day to proceed to ascribe to the mitochondria a function of mediating cellular respiration as their only and exclusive one.

REGAUD in 1909 advanced his ectosome theory according to which the mitochondria play the part of choosing and selecting substances from the surrounding cytoplasm, condensing them and transforming them in their interior into infinitely diverse products. The fundamental idea of this theory, it may be said, was incorporated by COWDRY into his surface film theory, which will next be treated, in a more advanced and concrete form.

COWDRY ('26) suggested the importance for cellular physiology of the interface between the mitochondria and the cytoplasm. That various substances may be adsorbed and concentrated on the surface film of the mitochondria may safely be inferred from the fact that these are stained vitally with Janus green B. Some of them, at least, e. g. starch or compounds containing masked iron, may be incorporated into the substance of the mitochondria. Probably some chemical or physical change or changes on either side may here be a necessary preliminary. Next will follow a series of chemical or physical interactions between the mitochondrial material and the incoming substances, and substances of widely different character may be built up. As there are variations in the chemical constitution of the mitochondria and also in that of the cytoplasm, different actions are conceived either in adsorption or presumably in catalysis. Thus, the mitochondria-cytoplasmic interface may be considered, COWDRY states, to play a rôle of as real importance in cellular physiology as the nuclear membrane.

In what part or parts of the cell chemical reactions necessary for the maintenance of vital phenomena are going on is one of the most important problems of cellular physiology. According to HOFMEISTER ('01), different chemical reactions in the cell go on in different ultramicroscopic reaction chambers bounded by semipermeable membranes, which are formed by the reactions themselves and continuously appear and disappear in the cytoplasm. Others, among whom WARBURG's ('21) name may be mentioned, are of the opinion that intracellular surfaces, surface films of various kinds of granules in particular, may be assigned the important rôle of adsorbing and concentrating molecules of various substances and thus facilitating chemical interactions. Ingenious is the theory of ultramicroscopic reaction chambers, but it is not supported by any positive evidence. On the other hand, the latter theory, which may be termed as one of surface catalysis, is based upon sound experimental facts and is provided also within the limits of the mitochondrial problem itself with positive evidence. Among other evidence, there is no room for doubt that Janus green B is absorbed and concentrated and becomes sometimes reduced on the surface of the mitochondria. HIRSCH's finding ('32), also, that the secretion granules make their first appearance on the surface of the mitochondria may perhaps be best interpreted on the basis of this theory, although, according to RIES ('35), they are no primordial secretion granules, but are composed of lipoidal



substances concentrated and appearing in granular form on the surface of mitochondria. Thus, in the present state of our knowledge, the surface film theory, which may be considered as essentially a theory of surface catalysis with regard to the mitochondria, seems to be the most plausible of many theories and hypotheses on the functional significance of the cytological constituents in question. The supposition may be allowed that other formed constituents of the cytoplasm may also function in a similar way but the mitochondria with others may probably have their respective sphere of activity assigned to them according to their physical and chemical properties. ROBERTSON ('26) expressed the opinion that the mitochondria adsorb and so orient molecules of some organic substances on their surface that reactive atomic groups draw near each other and become concentrated and chemical synthesis is facilitated. Such a consideration will contribute, no doubt, to refinement of the theory, but what is here more needed seems to be further accumulation of positive evidence.

In brief, the writer's opinion on the nature and functional significance of the mitochondria may be stated as follows. The mitochondria are present in all the animal cells except some which are at the terminal stages of ontogenetic history. They are to be regarded as constituting indispensable phases of the hyaloplasm in which they are embedded, differing, however, in consistency from the latter. In normal conditions, the number of mitochondria contained in unit volume of the cytoplasm seems to remain fairly constant in different cells of a definite type. There is some evidence in support of the view that, when the cell is injured so that it is deprived of its proper garniture of mitochondria, the cytoplasm is, in a sense, out of equilibrium and the cell cannot be functionally active. Why, in normal cells, the quantity of the mitochondria is definite in such a manner is a difficult problem which will await experimental analysis in future days. As regards their functional significance, it probably consists in surface catalysis in the sense which COWDRY indicates in his surface film theory. Though direct evidence in its favour is still meager on account of technical difficulties, it will gradually be accumulated by future studies.

One important point which I wish especially to emphasize is that the quantity of the mitochondria of a cell is determined primarily by the quantity of the cytoplasm and not by the demand for their functional activity. They may be regarded in this respect as constituting part of the organization of the protoplasm. This point has never come into the mind of most investigators. For example, it was thought by many that the mitochondria of a cell should increase or decrease according to whether their supposed functions are claimed or not. But mere demand in functional activities of a vital structure does not necessarily lead to an increase in number of its structural units. Who thinks that the nerve cells of the brain should increase during mental activities and decrease during hours of rest? There is no reason to surmise that in a single instance minute structural elements such as the mitochondria must fluctuate in number according to the degree of

their activity. It is a conclusion already arrived at that there is, in fact, no indubitable evidence in favour of the supposition that the quantity of the mitochondria should change according to the physiological conditions of the cell (p. 188).

### V. Summary

From the writer's own studies, outlines and results of which are comprised in Chap. II, from general theoretical considerations and from critical survey of literature, the following conclusions are arrived at:

1. Mitochondria are present in all healthy animal cells except some of those which are at the terminal stages of their ontogenetic history.

2. Mitochondrial configuration is nearly the same in different cells of a given kind pertaining to an individual or to different individuals of a given species of animal. Changes in it occur indubitably in some cases during the ontogenetic history or the functional cycle of the cell, but they are no necessary attendant of either of the latter.

3. The diameter or girth of all the mitochondria in one and the same cell is astonishingly uniform. Changes in it occur in some cases, but not always, during the ontogenetic history or the functional cycle of the cell. In such instances, all the mitochondria of the cell undergo similar and simultaneous changes.

4. The localization of mitochondria in the cell is known, at least in some cases, to be conditioned by the colloidal state of the protoplasm. Their orientation seems to be determined in many cases by the protoplasmic streaming, the diffusion or water current through the cell. It is evident that the presence of formed materials in the protoplasm, such as the GOLGI material, myofibrils, fat droplets etc., may act in some way and to some extent in modifying the localization and orientation of the mitochondria in the cell.

5. The mitochondrial content of a normal cell does not undergo quantitative changes to any marked degree during the functional cycle of the latter. On the contrary, it evidently increases or decreases in some cases during the process of cellular differentiation or senescence, being accompanied here, however, with simultaneous increase or decrease respectively in quantity of the cytoplasm. The number of mitochondria per unit volume of cytoplasm is much the same among different cells of the same type.

6. Mitochondria may be considered as representing indispensable morphological constituents of the cytoplasm. No particular substances, however, which do not exist in the hyaloplasm are believed to be contained in their chemical composition. Mitochondria may probably be interpreted as constituting merely particular phases of the hyaloplasm in which they are embedded, differing, however, in physical consistency from the latter.

7. The functional significance of mitochondria in the cell may, in all probability, consist in surface catalysis in the sense expressed in the surface film theory. It may be conceived that other formed constituents of the

cytoplasm may also participate in this, but the mitochondria and the others may probably have their respective sphere of activity assigned to them according to their physical and chemical properties. The supposition that the mitochondria of a cell should increase or decrease according to whether their functional participation is demanded or not, cannot be supported theoretically and also contradicted by a body of unquestionable evidence.

### Literature

- ALTMANN, R., 1890. Die Elementarorganismen und ihre Beziehung zu den Zellen. Leipzig.
- ARNOLD, G., 1912a. On the condition of epidermal fibrils in epithelioma. *Quart. J. Micr. Sc.*, Vol. 57, p. 283.
- , 1912b. The rôle of the chondriosomes in the cells of the guinea pig's pancreas. *Arch. f. Zellforsch.*, Vol. 8, p. 252.
- AVEL, M., 1924. Sur l'appareil de Golgi des hématies de Grenouille. *Compt. rend. Soc. d. biol.*, Vol. 90, p. 792.
- BENSLEY, R. R. and GERSCH, I., 1933. Studies on cell structure by the freezing-drying method. II. The nature of mitochondria in the hepatic cells of *Amblystoma*. *Anat. Record*, Vol. 57, p. 217.
- BENSLEY, R. R. and HOERR, N. L., 1934. Studies on cell structure by the freezing-drying method. VI. The preparation and properties of mitochondria. *ibidem*, Vol. 60, p. 449.
- CHAMBERS, R., 1915. Microdissection studies on the germ cell. *Science*, N. S., Vol. 41, p. 290.
- , 1924. The physical structure of protoplasm as determined by microdissection and injection. In "General Cytology." Chicago.
- CHAMPY, C., 1913. Recherches sur la spermatogénèse des batraciens et les éléments accessoires du testicule. *Arch. de zool. expériment. et gén.*, Vol. 52, p. 13.
- CHANG, H. C., 1931. Mitochondria in osteoclasts. *Anat. Record*, Vol. 49, p. 397.
- CIACCIO, C., 1913. Les plastosomes des éléments de la série hémoglobinique. *Folia haematol.*, Vol. 15, p. 391.
- CLARK, J. A. and HAIR, G. E., 1932. Observations on the mitochondria of the hepatic cell of the frog in normal and hyperglycemic states. *Zeit. Zellf. mikr. Anat.*, Vol. 15, p. 123.
- COWDRY, E. V., 1914a. The vital staining of mitochondria with Janus green and diethylsafranin in human blood cells. *Intern. Monatschr. f. Anat. u. Physiol.*, Vol. 31, p. 267.
- , 1914b. The comparative distribution of mitochondria in spinal ganglion cells of vertebrates. *Am. J. Anat.*, Vol. 17, p. 1.
- , 1918. The mitochondrial constituents of protoplasm. *Contrib. Embryo. Carnegie Inst. Wash.*, Vol. 8, p. 39.
- , 1923. The independence of mitochondria and the *Bacillus radicola* in root nodules. *Am. J. Anat.*, Vol. 31, p. 339.
- , 1924. Cytological constituents—mitochondria, Golgi apparatus, and chromidial substance. In "General Cytology." Chicago.
- , 1926. Surface film theory of the function of mitochondria. *Amer. Nat.*, Vol. 60, p. 157.
- , 1934. A Textbook of Histology. London.

- COWDRY, E. V. and COVELL, W. P., 1927. Quantitative cytological studies on the renal tubules. II. Mitochondria-cytoplasmic ratio. *Anat. Record*, Vol. 36, p. 349.
- COWDRY, E. V. and OLITSKY, P. K., 1922. Differences between mitochondria and bacteria. *J. Exper. M.*, Vol. 36, p. 521.
- COWDRY, N. H., 1917. A comparison of mitochondria in plant and animal cells. *Biol. Bull.*, Vol. 33, p. 196.
- CRAMER, W. and LUDFORD, R. J., 1925. On cellular changes in intestinal fat absorption. *J. Physiol.*, Vol. 60, p. 342.
- , 1926. On the cellular activity and cellular structure as studied in the thyroid gland. *ibidem*, Vol. 61, p. 398.
- DAWSON, A. B., 1928. Mitochondria and neutral-red bodies in the differentiating erythrocytes of *Necturus*. *Anat. Record*, Vol. 39, p. 137.
- DEJKUN, B., 1929. Zur Frage der Morphologie des Chondrioms bei der Sarkolyse. *Z. Zellforschg.*, Vol. 10, p. 135.
- DUESBERG, J., 1909. Über Chondriosomen und ihre Verwendung zu Myofibrillen beim Hühnerembryo. *Verhandl. d. Anat. Ges. zu Giessen*, p. 123.
- GAMBIER, E., 1929. Evolution du chondriome ordinaire et du chondriome actif (lépidosome) au cours de la spermatogénèse et la spermiogénèse du discoglosse. *Compt. rend. Soc. d. biol.*, Vol. 101, p. 667.
- GIROUD, A., 1929. Recherches sur la nature chimique du chondriome. *Protoplasma*, Vol. 7, p. 72.
- , 1931. Les substance à fonction sulfhydrile du protoplasma. *ibidem*, Vol. 12, p. 23.
- GOETSCH, E., 1916. Functional significance of mitochondria in toxic thyroid adenomata. *Johns Hopkins Hosp. Bull.*, Vol. 27, p. 129.
- Goss, C. M., 1930. Differentiation of mitochondria and granules by microdissection. *Anat. Record*, Vol. 45, p. 217.
- GRAY, J., 1931. *A Text-Book of Experimental Cytology*. London.
- GUILLIERMOND, A., 1918. Sur la signification du chondriome. *Rev. gén. bot.*, Vol. 30, p. 161.
- , 1927. Observations vitales sur l'instabilité de formes des mitochondries et leur permanence. *Bull. biol. de Fr. et Belg.*, Vol. 61, p. 1.
- , 1932. La structure de la cellule végétale: Les inclusions du cytoplasme et en particulier les chondriosomes et les plastes. *Protoplasma*, Vol. 16, p. 291.
- , 1934. Les constituants morphologiques du cytoplasme: Le chondriome. *Actualités scientifiques et industrielles* 170. Paris.
- GURWITSCH, A., 1913. *Vorlesungen über allgemeine Histologie*. Jena.
- HALL, E. M. and MAC KAY, E. M., 1933. The relation between the mitochondria and glucose-glycogen equilibrium in the liver. *Am. J. Path.*, Vol. 9, p. 205.
- HALL, R. P., 1929. Reaction of certain cytoplasmic inclusions to vital dyes and their relation to mitochondria and Golgi apparatus in the flagellate *Peranema trichophorum*. *J. Morph. a. Physiol.*, Vol. 48, p. 105.
- HIRSCH, G. C., 1931a. Die Restitution des Sekretmaterials im Pankreas. Eine Zusammenfassung meiner neueren Ergebnisse. *Verh. d. Deut. Zool. Gesells.*, Vol. 34, p. 302.
- , 1931b. Analyse der Restitution des Sekretmaterials im Pankreas mittels Röntgenstrahlen. *Roux' Arch.*, Vol. 123, p. 792.
- , 1932. Die Lebensbeobachtung der Restitution des Sekretes im Pankreas. I. Tl.: Die Restitution der Granula nach Pilocarpinreizung, ihr Ort und ihre Zeit. *Z. Zellforschg.*, Vol. 15, p. 36.

- HORNING, E. S., 1927. On the orientation of mitochondria in the surface cytoplasm of infusorians. *Austr. Jour. of Exp. Biol. a. Med. Sci.*, Vol. 4, p. 187.
- HOSSELET, C., 1929. Chondriome et appareil de Golgi dans les glands sericigènes des phryganides. *Compt. rend. Soc. d. biol.*, Vol. 101, p. 87.
- HOVEN, H., 1910. Sur l'histogénèse du système nerveux périphérique chez le poulet et sur le rôle de chondriosomes dans la neurofibrillation. *Arch. de Biol.*, Vol. 25, p. 427.
- , 1911. Du rôle du chondriome dans l'élaboration des produits de sécrétion de la gland mammaire. *Anat. Anz.*, Vol. 39, p. 321.
- IWASAWA, K., 1930. Experimentelle Forschungen über den Einfluss der ophthalmologischen Behandlungen auf den feineren Bau der Ziliarepithelzellen und über die Funktion derselben. *Acta Soc. ophthalm. jap.*, Vol. 34, p. 7.
- JOYET-LAVERGNE, Ph., 1923. Sur le rôle du chondriome dans le métabolisme cellulaire. *Compt. rend. Soc. d. biol.*, Vol. 97, p. 471.
- , 1923. Le pouvoir oxydo-réducteur du chondriome des grégaires et les procédés de recherches du chondriome. *ibidem*, Vol. 98, p. 501.
- , 1923. Sur le pouvoir oxydo-réducteur du chondriome. *Compt. rend. Acad. d. sc.*, Vol. 186, p. 471.
- , 1929. Glutathion et chondriome. *Protoplasma*, Vol. 6, p. 84.
- , 1932. Sur le pouvoir oxydant du chondriome dans la cellule vivante. *Compt. rend. Soc. d. biol.*, Vol. 110, p. 552.
- , 1933. Pouvoir oxydant, chondriome et sexualisation cytoplasmique chez les champignons. *Compt. rend. Acad. d. sc.*, Vol. 195, p. 894.
- , 1936. La démonstration expérimental du rôle du chondriome dans la respiration. *ibidem*, Vol. 203, p. 1291.
- JORDAN, H. E., 1914. Mitochondria and Golgi apparatus of the giant cells of red bone marrow. *Am. J. Anat.*, Vol. 29, p. 117.
- KATER, J. McA., 1923. Morphological aspects of protoplasmic and deutoplasmic synthesis in oogenesis of *Cambarus*. *Z. Zellforschg.*, Vol. 3, p. 186.
- , 1931. Variations in the mitochondria of the hepatic cell in relation to alterations of the glycogen-glucose equilibrium. *Anat. Record*, Vol. 49, p. 277.
- , 1937. The liver-blood fluid exchange and the morphology of hepatic cell mitochondria. *J. Morph. a. Physiol.*, Vol. 61, 473.
- KATŌ, Y. and NISIDA, S., 1935. Cytological studies on the anterior pituitary of the domestic fowl (Jap.). *Nippon-Tikusan-Gakkaihō*, Vol. 3, p. 16.
- KINGSBURY, B. F., 1912. Cytoplasmic fixation. *Anat. Recrd*, Vol. 6, p. 39.
- KITAMURA, Y., 1923. Changes in the fine structure of the intestinal villi accompanying absorptive activity in the rabbit (Jap). *Kaibō Z.* Vol. 1, p. 22.
- KURASHIGE, S., 1930. Zytologische Studien über die Erythrozyten beim japanischen Riesensalamander mit besonderer Berücksichtigung der physiologischen Bedeutung der „Golgi-Substanz.“ *Fol. anat. jap.*, Vol. 8, p. 137.
- LAGUESSE, E., 1926a. La première ébauche des fibrilles conjonctives provient elle du chondriome? *Arch. Anat. microsc.*, Vol. 22, p. 129.
- , 1926b. L'histogénèse des fibrilles de la cornée dans ses rapports avec le chondriome. *ibidem*, Vol. 22, p. 293.
- LÉGER, L. and DUBOSCQ, O., 1916. Sur les mitochondries du *Balantidium elongatum* Stein. *Compt. rend. Soc. d. biol.*, Vol. 79, p. 46.
- LEWIS, M. R., 1917. Development of connective-tissue fibres in tissue cultures of chick embryos. *Contrib. Embryo. Carnegie Inst. Wash.*, Vol. 6, p. 45.
- LEWIS, W. H., 1919. Degeneration granules and vacuoles in the fibroblasts of chick embryos cultivated in vitro. *Johns Hopkins Hosp. Bull.*, Vol. 30, p. 81.

- LEWIS, W. H. and LEWIS, M. R., 1915. Mitochondria and other cytoplasmic structures in tissue cultures. *Am. J. Anat.*, Vol. 17, p. 339.
- LIU, A. C., 1930. The mitochondria-Golgi complex of the columnar epithelium of the small intestine during absorption. *Chin. J. Physiol.*, Vol. 4, p. 359.
- LUDFORD, R., 1924. Cell organs during keratinization in normal and malignant growth. *Quart. J. Micr. Sci.*, Vol. 69, p. 27.
- MA, W. C. and PILLAT, A., 1929. A study of the function of the columnar epithelium of the ciliary body of albino rabbits. *Chin. J. Physiol.*, Vol. 3, p. 363.
- MAXIMOW, A., 1913. Über Chondriosomen in lebenden Pflanzenzellen. *Anat. Anz.*, Vol. 43, p. 241.
- MAYER, A., RATHERY, F., and SCHAEFFER, G. 1914. Sur les variations expérimentales du chondriome hépatique; parallélisme entre la composition chimique du tissu et ses aspects cytologiques. *Compt. rend. Soc. d. biol.*, Vol. 76, p. 393.
- MEVES, F., 1907a. Über Mitochondrien bezw. Chondriokonten in den Zellen junger Embryonen. *Anat. Anz.*, Vol. 31, p. 399.
- , 1907b. Die Chondriokonten in ihrem Verhältnis zur Filarmasse Flemmings. *ibidem*, Vol. 31, p. 561.
- , 1903. Die Chondriosomen als Träger erblicher Anlagen. *Cytologische Studien am Hühnerembryo*. *Arch. f. mikr. Anat.*, Vol. 72, p. 816.
- , 1909. Über Neubildung quergestreifter Muskelfasern nach Beobachtungen am Hühnerembryo. *Anat. Anz.*, Vol. 34, p. 161.
- MILOVIDOV, P. F., 1923a. Sur la question de la double coloration des bactéries et des chondriosomes. *Compt. rend. Soc. d. biol.*, Vol. 93, p. 555.
- , 1923b. Influence de la centrifugation sur les chondriosomes et les bactéries symbiotiques. *Arch. Anat. microsc.*, Vol. 24, p. 19.
- , 1933. Independence of chondriosomes from nuclear matter. *Cytologia*, Vol. 4, p. 158.
- MITAMURA, T., 1934. Morphoplasm of the cell: Mitochondria, metachondria, and Golgi apparatus (Jap.). *Iwanami-Kōza, Seibutu-gaku*.
- MORELLE, J., 1924. Les constituants du cytoplasme dans le pancréas et leur rôle dans la sécretion. *Bull. Acc. Belgique Cl. Sci.*, Vol. 9, p. 139. (Ref. Zool. Ber., Bd. 6, p. 346.)
- MUKAI, H., 1929. On the fine structure of the epithelial cells of the ciliary body of the rabbit (Jap.). *Nihon Ganka Gk. Z.*, Vol. 33, p. 241.
- NEEDHAM, J. and NEEDHAM, D. M., 1927. The oxidation-reduction potential of protoplasm. *Protoplasma*, Vol. 1, p. 255.
- NICHOLSON, F. M., 1923. Changes in the mitochondria produced experimentally in the thyroid gland. *J. Exp. Med.*, Vol. 39, p. 63.
- NISIO, H., 1931a. Experimental studies on the fine structure of the cell protoplasm. I. On the influence of osmotic pressure on the mitochondria and Golgi apparatus (Jap.). *Kaibō Z.* Vol. 4, p. 449.
- , 1931b. Experimental studies..... II. On the influence of different hydrogen ion concentrations on the mitochondria and Golgi apparatus (Jap.). *ibidem*, Vol. 4, p. 629.
- , 1931c. Experimental studies..... III. On the influence of radium rays on the mitochondria and Golgi apparatus (Jap.). *Nihon Röntg. Gk. Z.*, Vol. 9, p. 358.
- , 1932. Experimental studies..... IV. On the influence of Röntgen rays on the mitochondria and Golgi apparatus (Jap.). *ibidem*, Vol. 9, p. 613.
- NISIO, H. and HONDA, S., 1932. Experimental studies..... V. On the influence of Röntgen rays on the mitochondria. (Observation on vitally stained material) (Jap.). *ibidem*, Vol. 9, p. 641.

- OKADA, Y. K., TAKAGI, S., and SUGINO, H., 1933. Microchemical studies on the so-called photogenic granules of *Watasenia scintillans* (Berry). Proc. Imp. Acad., Vol. 10, p. 431.
- OKAMOTO, H., 1924. Über die Mitochondrien der Leber und Niere bei den Februar- und Maifröschen. Virchows Arch. f. path. Anat. u. Physiol., Vol. 250, p. 275.
- POLLISTER, A. W., 1932a. The cytology of the liver of *Amphiuma*. Am. J. Anat., Vol. 50, p. 179.
- , 1932b. The development of leucopoietic tissue in *Amblystoma punctatum*. Collecting Net, Vol. 7, p. 275.
- RADU, V., 1930. Le noyau générateur de mitochondries dans les cellules glandulaires du canal déférent chez *Armadillidium vulgare* Latr. Compt. rend. Soc. d. biol., Vol. 103, p. 285.
- RASMUSSEN, A. T., 1920. The mitochondria in nerve cells during hibernation and inanition in the woodchuck (*Marmota monax*). J. Comp. Neurol., Vol. 31, p. 37.
- REGAUD, C., 1909. Sur la signification physiologique du chondriome des cellules sexuelles mûres, et notamment des spermatozoïdes. Compt. rend. Soc. d. biol., Vol. 67, p. 443.
- REGAUD, C. and FAVRE, M., 1912. Nouvelles recherches sur les formations mitochondriales de l'épiderme humain, a l'état normal et pathologique. ibidem, Vol. 72, p. 328.
- RIES, E., 1935. Zur Histologie des Mäusepankreas nach Lebendbeobachtung, Vitalfärbung und Stufenuntersuchung. Z. Zellforsch., Vol. 22, p. 523.
- ROBERTSON, B., 1926. The function of the lipid in mitochondria. Austr. Jour. of Exp. Biol. a. Med. Sci., Vol. 3, p. 97.
- ROMEIS, B., 1913. Das Verhalten der Plastosomen bei der Regeneration. Anat. Anz., Vol. 45, p. 1.
- SAGUCHI, S., 1920. Studies on the glandular cells of the frog's pancreas. Am. J. Anat., Vol. 26, p. 347.
- , 1927. Untersuchungen über die Wechselbeziehung zwischen Karyo- und Cytoplasma. I. Zentronephelium und Chondriom, und ihre Beziehung zum Kern, nebst einem Beitrag zur Frage nach der Bedeutung der Nucleolen. Beobachtungen an den explantierten Geweben des Hühnerembryos. Zytol. Studien, Heft 1.
- SAITŌ, R., 1933. Study on mitochondria and metachondria of intestinal epithelial cells. Jap. J. of exper. Med., Vol. 11, p. 397.
- SMITH, D. M., 1931. The ontogenetic history of the mitochondria of the hepatic cell of the white rat. J. Morph. a. Physiol., Vol. 52, p. 485.
- TAKAGI, S., 1929a. Über das Verhalten der Chondriosomen in den verschiedenen Organen der Kröte beim Winterschlaf und Hungerzustand. Nagoya J. med. Sci., Vol. 4, p. 38.
- , 1929b. Über die Chondriosomen in dem elektrischen Organe bei *Narke japonica*. ibidem, Vol. 4, p. 70.
- , 1929c., Über die Chondriosomen in den Purkinjeschen Zellen des Reizleitungssystems im Herzen. ibidem, Vol. 4, p. 72.
- , 1931. Studien über die Differenzierung der Blutzellen, beobachtet aus dem Standpunkt der Chondriosomenforschung. Hämatopoese im Dottersack des Hühnerembryos. Annot. Zool. Japon., Vol. 13, p. 105.
- , 1932a. Über das Verhalten der Chondriosomen bei der mitotischen Zellteilung der jüngeren Blutzellen. ibidem, Vol. 13, p. 355.
- , 1932b. Studies on mitochondria (Jap.). Zool. Mag. (Japan), Vol. 44, p. 9.
- , 1932c. On the mitochondria in *Dicyema* (Jap.). ibidem, Vol. 44, p. 361.

- TAKAGI, S., 1933a. Einige Beobachtungen an den Chondriosomen bei Zyklostomen, *Entosphenus japonicus* (Martens). Annot. Zool. Japon., Vol. 14, p. 89.
- , 1933b. Mitochondria in the luminous organs of *Watasenia scintillans* (Berry). Proc. Imp. Acad., Vol. 9, p. 651.
- , 1934a. Mitochondria in the luminous organs of *Luciola cruciata* Motschulsky. ibidem, Vol. 10, p. 692.
- , 1934b. A treatise on mitochondria. I. On the quantity and changes in quantity of the mitochondria of the cell (Jap.). Syokubutu oyobi Dōbutu, Vol. 2, p. 865.
- , 1934c. A treatise on mitochondria. II. On the form and changes in form of the mitochondria (Jap.). ibidem, Vol. 2, p. 1507.
- , 1935. A treatise on mitochondria. III. On the arrangement of the mitochondria in the cell (Jap.). ibidem, Vol. 3, p. 910.
- , 1936a. Über Sekretbildung in dem Leuchtorgan von *Cypridina hilgendorfi* Müller, mit besonderer Berücksichtigung der Mitochondrien. Annot. Zool. Japon., Vol. 15, p. 344.
- , 1936b. Cytoplasmic inclusions of the erythrocyte of *Triturus pyrrhogaster* (Boie) (Jap.). Syokubutu oyobi Dōbutu, Vol. 4, p. 142.
- , 1939. A quantitative study of mitochondria in some ciliates (Jap.). Zool. Mag. (Tokyo), Vol. 51, p. 124.
- THURLOW, M. D., 1916. Observations on the mitochondrial content of the cells of the nuclei of the cranial nerves. Anat. Record, Vol. 10, p. 253.
- , 1917. Quantitative studies on mitochondria in nerve cells. Contrib. Embryo. Carnegie Inst. Wash., Vol. 6, p. 35.
- TIRELLI, M., 1923. Modificazioni del condrioma e del lacunoma nelle cellule intestinali di "*Gambusia holbrooki*" durante le diverse fasi dell'attività funzionale e durante il digiuno. (Ref. Ber. ii. d. wiss. Biol., Vol. 8, p. 338.).
- TSUKAGUCHI, R., 1926. On the structure of protoplasm (Jap.). Nippon-Gakuyutu-Kyōkai Hōkoku, Vol. 2, p. 449.
- UETANI, T., 1933. Über die Mitochondrien im Knochenmarksgewebe des menschlichen Sternalpunktaten. Nagoya J. med. Sci., Vol. 12, p. 117.
- VOLKONSKY, M., 1933. Digestion intracellulaire et accumulation des colorants acides. Étude cytologique des cellules sanguines des Sipunculidés. Bull. biol. de Fr. et Belg., Vol. 97, p. 135.
- WALLGREN, A., 1911. Zur Kenntnis der Plasmastruktur der Plasmazelle. Beitr. z. path. Anat. u. z. allg. Pathol., Vol. 51, p. 227.
- WALLIN, I. E., 1923. The mitochondria problem. Am. Nat., Vol. 57, p. 255.