EXPERIMENTAL BIOLOGY

ON THE ROLE OF DESOXYRIBONUCLEIC ACID IN THE VERTEBRATE FERTILIZATION PROCESS

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The investigation was carried out on representatives of various classes of vertebrates. The gonads of 7 bony fish (golyana), of 19 anurous amphibians (green toads), of 8 caudate amphibians (4 crested salamanders and 4 axolotls), 10 birds (5 sparrows, 2 strixes, 3 wild teal ducks), 9 mammals (6 white mice, 3 cats) and 3 humans. The preparations were stained by means of the Feulgen reaction to demonstrate desoxyribonucleic acid (DNA).

In investigating the testes, we observed that the nuclei of the spermatozoa were completely saturated with DNA in all cases. At the same time the nuclei of the spermatogonia and spermatocytes stained more faintly and contained DNA in smaller concentrations than the spermatozoa. During the process of spermatogenesis, the concentration of DNA increased especially sharply in the spermatids and spermatozoa in the progression of "spermatogonia-spermatocytes-spermatids-spermatozoa".

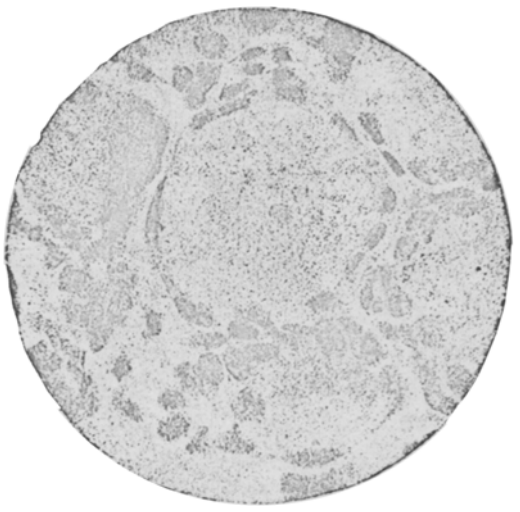

Fig. 1. Tissue from the gonad of the tadpole of the green toad. Desoxyribonucleic acid is absent in the nuclei of the oocytes.

During investigation of the ovaries of sexually mature fish, amphibians and birds, the absence of DNA was noted in the nuclei of the oocytes. Along with this, we observed that in three preparations in which the oocyte nuclei did not stain at all by Feulgen's method, the nuclei of the follicular cells surrounding the oocytes stained intensively and specifically, indicating that they contained a considerable amount of DNA.

At early stages of embryogenesis in the caudate amphibians (axolotl) and anurous amphibia (green toad), the oocyte nuclei contained DNA, but in considerably smaller concentrations than the nuclei of the follicular cells surrounding them. At later stages of development of the caudate amphibians, the DNA content of the oocyte nuclei decreased and by the end of metamorphosis the oocyte nuclei did not stain at all when Feulgen's reaction was carried out (DNA was lacking in them). The nuclei of the follicular cells surrounding the oocytes stained intensely and, consequently, contained DNA in considerable concentrations.

When studying the ovaries of mammals it was found that oocytes at the graafian follicle stage do not contain DNA, while the nuclei of the follicular cells surrounding them contain a considerable amount of this substance.

In the nuclei of oocytes an insignificant amount of DNA is contained at the primary follicle stage, at a much lower concentration than in the nuclei of the follicular cells surrounding them. A large amount of DNA is contained in the nuclei of the connective tissue cells of the gonadal stroma (Fig. 1, 2).

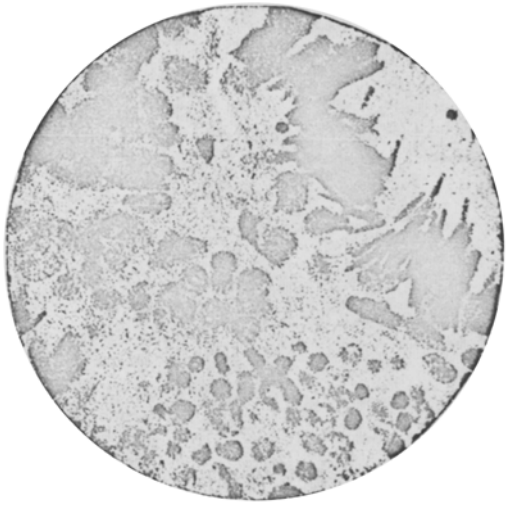

Fig. 2. Testicular tissue of the green toad. Nuclei of the spermatozoa are saturated with desoxyribonucleic acid.

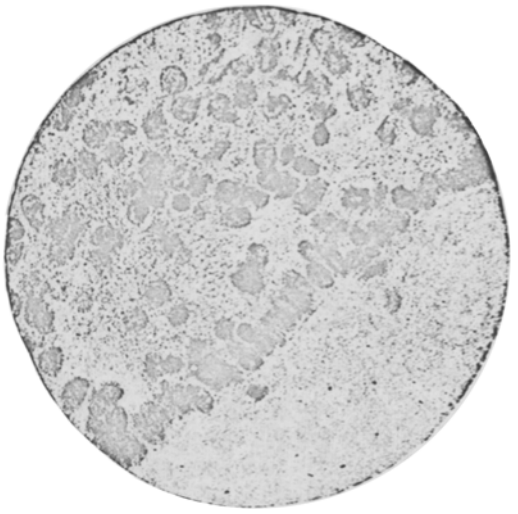

Fig. 3. Medulla oblongata of a green toad tadpole. The chromosomes of the cells undergoing division are saturated with desoxyribonucleic acid.

From the above data, the following can be concluded:

1) Mature male and female mammalian germ cells differ sharply from each other in the DNA content of the nuclei; the nuclei of spermatozoa are completely saturated with DNA, while DNA cannot be found in the nuclei of mature oocytes.

2) During the process of spermatogenesis and oogenesis, contradictory phenomena are observed; the DNA content in the male germ cells increases, reaching a maximum in the spermatozoa; in the female germ cells, the DNA concentration decreases, reaching such an insignificant amount that it cannot be found with Feulgen's reaction.

3) During the fertilization process of mammals, the spermatozoon brings DNA into the egg cell, which, probably, has great significance, especially for the beginning of cell division.

We can express a definite hypothesis regarding the role of DNA in fertilization on the basis of our own data and that found in the literature.

In studying the metamorphosis of anurous amphubians, the author [8] observed that during the period of karyokinesis in the chromosomes, DNA is accumulated.

When the nuclei of the various tissue cells are compared [8] in preparations stained by Feulgen's method, it becomes clear that they contain different concentrations of DNA. It is interesting to note that the more DNA there is in the nuclei, the smaller the nucleoli are and vice versa. The tissue cells can be divided into 5 groups according to the size of the nucleoli and the concentration of DNA, of which the second and third groups are little differentiated from each other.

First group. Nuclei maximally saturated with DNA, the nucleoli cannot be located. Spermatozoa are included in this group.

Second group. Nuclei contain DNA in large concentrations. Nucleoli small, 0.8-1μ in diameter. Histocytes, the lymphoid cells of the thymus, the endothelium of the capillaries, the epithelium of the renal corpuscles, the epithelium of the thyroid gland, of the olfactory organ, the smooth muscle cells, the neuroglia are included in this group.

Third group. The nuclei contain DNA in smaller concentration than the nuclei of the preceding group; the diameter of the nucleoli is 1.5-2μ. The striated muscle fibers, the intestinal epithelium, the epithelium of the renal tubules, the cutaneous epithelium, the hepatic epithelium, epithelium of the salivary glands and of the efferent ducts of the exocrine portion of the pancreas are included in this group.

Fourth group. Nuceli are poor in DNA, the nucleoli are large, 2.5-4μ in diameter. The large neurons of the spinal cord and medulla oblongata, the neurons of the spinocerebral ganglions, the secretory cells of the exocrine sections of the pancreas belong in this group.

Fifth group. The nuclei do not contain DNA, the nucleoli are very large, 10-11μ in diameter. The ovarian oocytes compose this group.

Fig. 4. Tissue from the tail of a green toad tadpole. Nuclear bodies, arising from the disintegrating nuclei, are saturated with DNA.

If the tissue cells of the above groups are analyzed from the point of view of frequency of mitotic division found among them, it is found that mitosis is encountered more frequently in the groups with large concentrations of DNA in the nuclei. On the contrary, the poorer the tissue cells are in DNA, the more rarely mitosis is encountered among them. The spermatozoa are an exception in this case, which can be explained by the fact that the DNA in their nuclei is in an inactive bound state until fertlization [18].

In investigating the organs of amphibians which undergo considerable destructive changes (tail, intestinal tract) during the period of metamorphosis, it was discovered that the DNA concentration increased in the nuclei not only in connection with cell division, but also in connection with the destruction of the cells during pycnotic changes in the nuclei. On the basis of the above, the hypothesis was made by us that DNA is a stimulator of biochemical reactions which occur both during cell division as well as during their destruction. In connection with this hypothesis, another was stated according to which the spermatozoa introduce DNA into the egg cell during fertilization, stimulating the process of cell division [8, 9].

In studying the literature on this problem, we found that many authors, even in old investigations, indicated the important role of the spermatozoa in the beginning of cell division; some considered that the spermatozoon brings the cellular center into the egg cell, others said that the sperm acted catalytically during contact. In the work of the last few years, indications can be found that the changes which occur during fertilization and cell division are dependent on the secretion of some kind of active substances by the chromosomes or head of the spermatozoon.

The authors of other papers speak of a change in the DNA content after fertilization, but do not connect this with the DNA in the spermatozoon. Swift and Kleinfeld [23] indicate the doubled amount of DNA in the nuclei of the segmenting eggs of the locust. Ludwig [19] described the change in DNA during the segmentation process in rats and khomyaks. During the prophase of segmentation, the author noted an accumulation of DNA, while the DNA disintegrated after the completion of cell division. At the 8-cell stage, the greatest amount of DNA was found in the nuclei. As the DNA content of the nuclei increased, a decrease in the size of the nucleoli was noted, which corresponds with our data also [8].

P. V. Makarov [4, 5], who studied the fertilization process of equine ascarids, described first a decrease, then an increase in the DNA content of the nuclear proteins. Braden and Austin [14] noted an increase in the DNA concentration during segmentation in rats. Brashe [13], investigating the development of amphibia, showed that 90 hours after fertilization the amount of DNA increased 26 times in the egg. All the above investigations confirm our views regarding the significance of DNA in the segmentation process.

The work of Horstadius [17] provokes special interest. When DNA was injected inside the unfertilized ovum of the sea urchin and it was mixed with the cytoplasm of the ovum, the ovum was activated and formed a fertilization membrane; Horstadius also notes that at low DNA concentrations the fertilization membrane is not formed.

In a number of papers there are indications that DNA is the active stimulator of the cell division processes both during segmentation as well as under other conditions.

Lison and Pasteels (cited from Zh. Brashe [12]) communicate that the DNA content increases in areas of intensive cell division.

K. Lang [18] writes that the formation of DNA occurs only during cell division. A. Bendich [11] and Zh. Shmerling [10] note that the intensity of the DNA metabolism increases during regeneration.

P. V. Makarov [5] noticed a regular increase of DNA in the nucleus during prophase and a decrease of it during telophase.

With respect to the DNA content of the germ cell nuclei, the literature data vary somewhat.

Pollister [21] indicates that the haploid nucleus of the spermatozoon and egg cell contains half as much DNA as the nuclei of somatic cells. The DNA content of one type of somatic cell in the organism is the same.

Dalcq [15] notes that DNA is found in a considerable concentration in the nuclei of the somatic cells of the mammalian ovary, including also the nuclei of the follicular cells. In the oocyte nuclei, according to A. Dalcq, the DNA is localized in the finest chromosomes.

Our own data, presented above, indicate that DNA is distributed differently in the nuclei of the various tissue cells of one species of animals (anurous amphibia) which does not correspond with the data of a number of other authors. At the same time, our data regarding the various distribution of DNA in the tissue cells found confirmation in some papers.

Pasteels and Bullough [20] noted that there is less DNA in the superficial cells of the epidermis of mice than in the lymphocytes, and that the DNA concentration in the growing layer of the epidermis is higher on the average than in the lymphocytes.

Zh. Brashe [12] cites the investigations of Lison and Pasteels (1951) of the change in the DNA content during the ontogenesis of the sea urchin. According to the data of these investigators, marked differences appear between the nuclei of various tissue cells with respect to DNA content.

Thomson and Frazer [24] note that 8 times as much DNA can be present in the rapidly regenerating liver nuclei than in normal ones.

Our data indicate that during the course of spermatogenesis and oogenesis, the DNA content of the nuclei changes in different ways, and not in the same way, as confirmed in some of the papers cited above.

According to our data, DNA, which is necessary for fertilization and egg segmentation, is introduced by the spermatozoon, whose nucleus contains it in maximum concentration. In some work it is possible to find confirmation of our data. Thus, D. Devidson [16] notes that DNA can compose 48.5% of the dry weight of the fat-free fraction of the sperm heads. P. V. Makarov [6] indicated that the sperm heads consist of nucleoprotein, which is in a condensed state, and are not packages of chromosomes. B. V. Kedrovsky [3] supplies data on the high DNA content of the nuclei of young spermatocytes of the grasshopper.

The feasibility of parthenogenetic development, in which segmentation occurs but DNA is not introduced into the egg cell from outside, contradicts at first glance our point of view regarding the role of DNA in the fertilization process of vertebrates. However, the role of DNA cannot be denied on this basis, as the role of the spermatozoon in the fertilization process cannot be denied just because it does not participate in parthenogentic reproduction.

It is possible to suggest that in the parthenogentic process, a change of RNA to DNA occurs in the egg cell, which stimulates segmentation. At the present time there are works in which the possiblity of changing RNA into DNA under certain conditions has been demonstrated. Thus, as early as 1931, Brashe [12] showed that during the development of sea urchin eggs an increase in the DNA content occurs while the cytoplasmic RNA decreases. G. I. Roskin [7] described the change of RNA into DNA during mitotic division.

Zh. G. Shmerling [10] states that disappearance of mitoses leads to a decrease in the amount of DNA in the tissues and to an increase in the RNA content.

B. I. Goldshtein and co-workers [1, 2] established the change of RNA into DNA in the tissues of the sea urchin outside of the organism under the influence of Vitamin C.

On the basis of all the above, the following can be stated: DNA is a chemical compound which stimulates biochemical reactions which occur during cell division. During fertilization, the spermatozoon introduces DNA into the egg cell, which creates in the zygote the DNA concentration necessary to stimulate the segmentation processes. In the case of parthenogentic reproduction, an increase in the DNA concentration at the expense of the RNA in the egg cell under the influence of external agents may be hypothesized.

SUMMARY

It was established that the amount of DNA in the nuclei of the cells of male gonads of mature fish, amphibia (Anura and Urodela), birds, mammals and man greatly increased in the process of spermatogenesis. Especially great amounts of DNA were found in the nuclei of spermatozoa. In mature oocytes, in contrast with the follicular cells, no DNA was found. The author believes that DNA, which plays an important part in the development of the fertilized egg is brought into the oocytes by spermatozoa.

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