CHANGE OF NUCLEIC ACID CONTENT IN STRUCTURAL COMPONENTS OF THE OVARIES OF WHITE RATS FED DIETS VARYING IN PROTEIN CONTENT

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In earlier published works [2], we discovered the structural and functional changes in ovaries which resulted from prolonged protein starvation followed by a well-balanced diet. The changes resulting from the starvation were expressed by growth retardation, delayed follicular differentiation and intensification of follicular atresia at the intermediate stages of follicular development, which led to a cessation of sexual development in immature rats and, in mature rats, to a cessation of the estrual cycle. In the ovaries of the starved rats, the primordial follicles were better preserved than the other structural components. After the animals had been transferred to a well-balanced diet, the structure and function of the ovaries was restored.

The purpose of this work was to study the changes in the ribonucleic and desoxyribonucleic acids in the structural components of ovaries in rats which had first undergone prolonged protein starvation and then been put on a balanced diet.

The studies were done on immature rats weighing 45-55 g and on sexually mature rats weighing 140-156 g. A total of 150 rats were used. The animals were kept on a synthetic diet, with a 19.5% protein content in the control and a 1.5% protein content in the experimental rats. The fat, vitamin and mineral content of both diets was the same. The immature rats were kept on the protein-deficient diet for a period of 23-64 days, the mature rats, for 45-135 days.

After long protein starvation, 57 rats (mature and immature) were put on the control diet. The immature rats were sacrificed on the 4th, 7th, 14th, and 17th days after the change of diet, and the mature rats, on the 14th, 25th and 50th days.

The animals were all sacrificed by decapitation; the mature rats were always killed during the diestrus period. The ovaries were fixed in Zenker-formol and by Geili's method, and the material was imbedded in paraffin. Ribonucleic acid (RNA) was determined by Brashe's method; sections 6 microns thick were stained with methyl green-pyronine according to Unna-Pappenheim, and some preparations were first processed with ribonuclease and then stained in the same way. Desoxyribonucleic acid (DNA) was shown by Feigen's reaction, with the preparations first stained light green.

Examination of the ovarian sections from the control rats, mature and immature, showed that the oocyte of the primordial follicle contained a large, bladder-like nucleus, rich in DNA, which was distributed in granules around the nucleolus, the nuclear membrane and on the threads of the linin net. One, or occasionally two nucleoli, which stained well with pyronine, were usually distributed eccentrically in the nucleus; the oocyte cytoplasm contained a considerable amount of RNA.

In the follicular cells, DNA was distributed over the entire nucleus in the form of large granules. The

nuclei contained one roundnucleolus, rich in RNA. The cytoplasm of these cells contained much RNA (Fig. 1,a).

DNA distribution in the oocyte nucleus changed according to the degree of growth and maturity of the primordial follicles and their transformation into young follicles; there were many small as well as large granules of DNA which were centrally placed around the nucleole, or sometimes more to one side of it. The space nucleolus around the nuclear membrane was usually free of DNA.

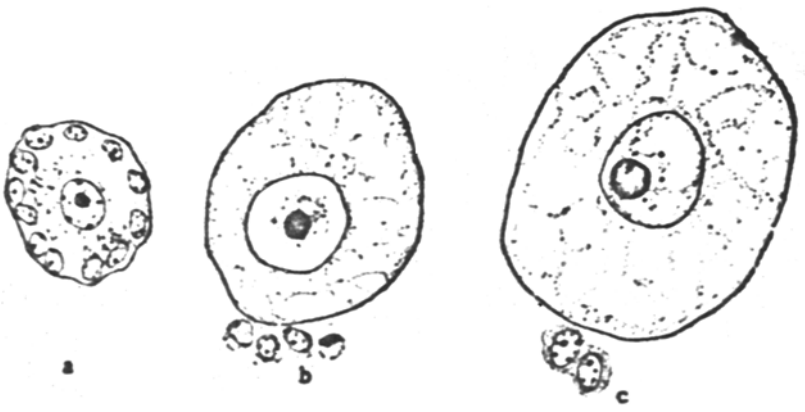

Fig. 1. Structural components of ovary in a control rat. Stained with methyl green-pyronine.
a) primordial follicle; b) oocyte of young growing follicle; c) oocyte of graafian vesicle.

The nucleolus was round, placed either in the center or to one side of the nucleus, and sometimes two nucleoli were observed; there was less RNA in the nucleolus than in the primordial follicles. There was either no RNA in the cytoplasm, or only traces of it around the nucleus and periphery of the oocyte.

The nuclei of the follicular cells in the young growing follicles were larger, rounder and richer in DNA, which was distributed throughout the nucleus in large granules. The cytoplasm of the follicular cells contained a considerable amount of RNA (Fig. 1,b).

The amount of DNA in the nucleus changed sharply during the process of growth and differentiation of the young follicle and its transformation into a graafian vesicle; the amount of RNA in the nucleolus decreased, and there was no RNA in the cytoplasm.

We did not observe any material changes in DNA distribution in the nuclei of the follicular cells nor in the amount of RNA in the nucleolus and cytoplasm of these cells but the DNA granules did become larger (Fig. 1,c).

The interkinetic nuclei of the cells of the young corpora lutea contained a small quantity of DNA – small lumps and granules of DNA were distributed throughout the entire nucleus. The lutein cells nucleoli contained a large amount of RNA and stained intensively with pyronine. One could observe the nucleoli coming out into the cytoplasm of the lutein cells, a process which, when observed completely, began with the approach of the nucleolus to the nuclear membrane and ended with its complete escape into the cytoplasm.

The cytoplasm of the young corpora lutea cells contained a small amount of RNA, which was mainly distributed around the nucleus. Some of the young corpora lutea cells were mitotically dividing, and the nuclei of such cells were rich in DNA, but their cytoplasm contained less RNA than the cytoplasm of the cells whose nuclei were interkinetic.

In the regression stage of the corpus luteum, its cells became small, the amount of DNA in its nuclei

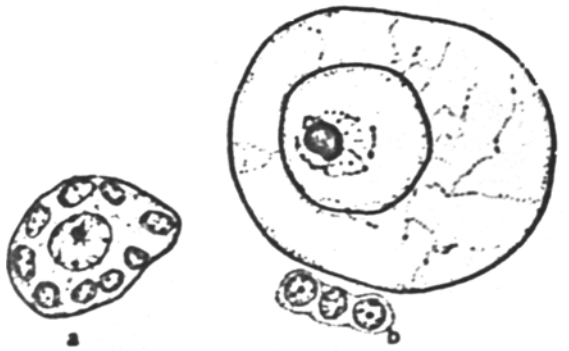

Fig. 2. Structural components of a rat's ovary after protein starvation. Stained with methyl green-pyronine. a) primordial follicle; b) oocyte of young follicle.

decreased, the nucleoli stained weakly with pyronine and RNA either completely disappeared from the cytoplasm or remained in a small amount.

Prolonged protein starvation causes a series of changes in the structural components of ovaries in mature and immature rats. These changes were of the same type, and we shall therefore give a general description of them below.

The DNA granules in the nucleus of the primordial follicle oocyte became coarser and were principally distributed around the nuclear membrane.

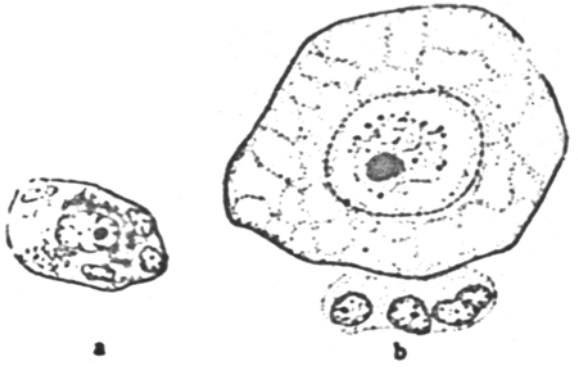

Fig. 3. Structural components of a rat's ovary during the restoration period. Stained with methyl green-pyronine. a) primordial follicle; b) young growing follicle.

The amount of RNA in the nucleolus and cytoplasm of the oocyte became somewhat smaller than the amount found in the primordial follicle oocyte from the control rat's ovary, but RNA did not, however, completely disappear.

In the nuclei of the follicular cells, DNA was distributed in coarser granules throughout the nucleus; the nucleolus and cytoplasm of the follicular cells contained less RNA and began to stain less intensively with pyronine (Fig. 2, a).

The following changes were observed in the oocytes of the young follicles: the DNA granules became larger and were mainly concentrated around the nucleolus; the amount of RNA in the nucleolus decreased; there was no RNA evident in the cytoplasm.

The DNA granules in the nuclei of the follicular cells became larger than the cells of normal ovaries. The cytoplasm and nucleoli of the follicular cells contained little RNA (Fig. 2, b).

In spite of the prolonged protein starvation, many follicular cells of the young follicles appeared in a state of karyokinetic division.

There were no undamaged mature graafian vesicles found in the ovaries of the starved rats.

During protein starvation, a series of changes also occurred in the corpora lutea of the mature rats. The amount of DNA granules in the nuclei of the lutein cells decreased, the nucleolus contained a small quantity of RNA and no RNA was found in the cytoplasm of the lutein cells.

All the corpora lutea of the starved rats were in the regression stage.

Consequently, protein starvation causes the quantity of RNA to decrease in the nucleoli and cytoplasm of all the ovarian structural components studied. One must note that the changes mentioned were most weakly expressed in the primordial follicles, which were better preserved than the other structural components in the ovaries of immature and mature rats.

After prolonged protein starvation, some of the animals were switched to a diet rich in protein.

The ovarian structural components of the immature rats changed considerably after 4 days of the balanced diet. The amount of DNA in the nucleoli and cytoplasm of the primordial follicle oocytes became greater than that in the primordial follicles of the ovaries in the starved rats.

The nuceli of the follicular cells of the primordial follicles retained the large, coarse granules of DNA, and the amount of RNA in the cytoplasm and nucleoli of these cells returned to normal (Fig. 3, a).

In the young, maturing follicles, the amount of RNA in the nucleolus and cytoplasm of the oocyte increased.

The nuclei of the follicular cells still retained the coarse granules of DNA, and the amount of RNA in the nucleoli and cytoplasm of these cells increased (Fig. 3, b).

The maturing primordial follicles and the graafian vesicles in the ovaries of immature rats which had been put on a balanced diet after protein starvation (balanced diet for 14-17 days) were morphologically and cytologically the same as the corresponding structural components in the ovaries of the control animals.

The study of ovarian structure in mature animals put on a balanced diet for a period of 4-7 days following protein starvation showed a picture essentially the same as that found in the ovaries of the starved animals; the gonads were therefore studied at later dates after the restorative diet had begun (14th, 25th and 50th days).

After 14 days of the balanced diet, a series of changes was observed in the cytological structure of the ovarian structural components in mature, starved rats. For example the amount of RNA in the nucleolus and cytoplasm of the primordial follicle oocytes increased to the amount found in the control rats.

The amount of RNA also returned to normal in the cells of the follicular epithelium.

As to DNA distribution in the nuclei of the oocytes and follicular epithelium of the young follicles, the ovaries of the experimental rats were the same as those of the control rats. The nucleoli contained a large amount of RNA.

When we examined ovarian sections from mature animals which had been put on a balanced diet after long protein starvation (duration of experiment: 25 days), we found that the ovarian structure and cytoplasm properties of these rats were the same as in the control animals.

Normal oogenesis was restored in the ovaries of the mature rats after 50 days of the balanced diet. Estrus

was observed in all of the animals 3-4 times during this period.

In the ovaries of rats fed a balanced diet for 25-50 days, the phenomenon of nucleolar escape from the nucleus was observed in the lutein cells; moreover, it was of a massive character.

The phenomenon of nucleolar escape which was observed in the corpus luteum cells could be considered as the manifestation of intense nucleic acids exchange between the nucleus and cytoplasm, which, in its turn, indicates the intensification of protein synthesis in the lutein cells.

The phenomenon of nucleolar escape was observed by Belousova [1] in the liver cells of rats fed a balanced diet after protein starvation.

As our studies have shown, long protein starvation causes the amount of DNA in the nuclei and the amount of RNA in the nucleoli and cytoplasm of the corpora lutea and of the follicles at various stages of maturity to decrease which indicates the decrease of nucleoprotein exchange in the ovarian structural components.

When a balanced diet is restores, so is the nucleoprotein exchange in the rats' ovaries, which is expressed by the increased amount of DNA and RNA in the cells of the structural components.

The cytological changes caused by protein starvation are less apparent in the primordial follicles, where the amount of DNA and RNA returns to the normal sooner than in the other structural components of the ovary.

SUMMARY

In sexually mature and immature rats kept for a long period of time on a protein-deficient diet (1.5% of protein) a fall of nucleoprotein metabolism in the structural components of the ovaries was observed. It was manifested by a decrease in the quantity of DNA in the nuclei and RNA in the cytoplasm and nucleoli of the primordial graafian follicles and corpus luteum. Cytologic changes were less manifested in primordial follicles.

When the rats were subsequently put back on a balanced diet (19.5% of protein) the quantity of DNA and RNA in the cells of structural components rose to normal, and the nucleoprotein metabolism in the ovaries was restored.

LITERATURE CITED

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[2] Z. A. Ryabinina, Doklady Akad. Nauk SSSR, 1952, Vol. 36, No. 4.