

# FUNCTIONAL AND STRUCTURAL CHANGES IN THE OVARIES OF RATS FED A WELL-BALANCED DIET AFTER BEING KEPT ON A PROTEIN-DEFICIENT DIET

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It has recently been established that the normal function of the ovaries is easily disturbed by different unfavorable influences on the body—by the toxic effect of various poisons [1, 4], during infectious diseases [2, 8], by the action of roentgenrays [9, 3], or by deficient or unbalanced diets.

Chronic starvation (partial or protein) delays sexual maturation in young animals [6, 7, 10] and suspends sexual development in adult animals [11].

Some authors have observed atresia intensification in the ovaries of starved animals and considerable connective tissue growth [7, 12, 13] as well as functional disturbance of the gonads.

However, the dynamics of the destructive changes occurring in the ovary during long protein starvation and the question of whether the disturbed function and structure of the ovary can be restored especially have not yet been sufficiently investigated. In an earlier paper [5], we established that long protein starvation causes the ovaries to decrease in weight and their function and structure to be disturbed.

This work presents data concerning the changes observed in the ovaries of animals which were fed a balanced diet after long protein starvation.

## EXPERIMENTAL METHODS

The experimental animals were fed synthetic diets—the control diet, with a 19.5% protein content and the experimental, with a 1.5% protein content. The fat, vitamin and mineral contents were the same in both diets.

The experiments were done on sexually immature (45-55 g) and sexually mature (140-156 g) rats.

The immature rats were fed a protein deficient diet for 45-64 days, during which time they lost 29-37% in weight. The mature rats were kept on the diet with the 1.5% protein content for 90-135 days. During this period, their weight decreased 35-53%.

After long protein starvation, some of the animals from each group were transferred to the control diet (19.5% protein). The immature rats were sacrificed on the 4th, 7th, 14th and 17th days of the balanced diet and the mature, on the 14th and 50th days.

All of the animals were sacrificed during the inactive phase of the sexual cycle. The ovaries were weighed in torsion scales and fixed in Zenker-formol fluid. The material was imbedded in paraffin. A series of sections 8 microns thick were prepared. The preparations were stained with hematoxylin-eosin. The ovarian structural components were counted in every fifth section (primordial follicles, follicles at different stages of maturity,

atretic follicles, corpora lutea and follicles with destroyed oocytes).

The results of the computations and the data on the relative weight of the ovaries were processed statistically.

## EXPERIMENTAL RESULTS

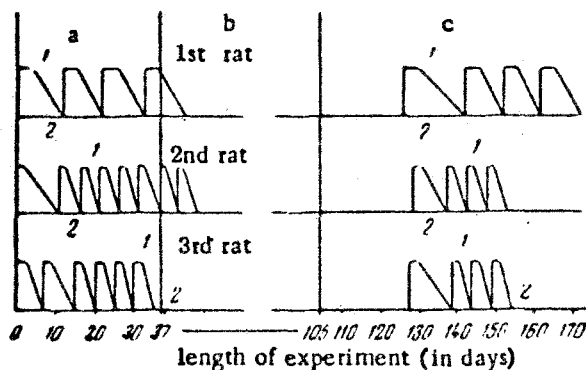
The absolute weight of the ovaries in all the rats fed the control diet after long protein starvation was greater than the weight of the ovaries in the starved animals (Tables 1 and 2).

The relative weight of the ovaries in the immature rats increased during the first 4-7 days of the balanced diet, then decreased and became even somewhat less than the relative weight of the gonad in the animals before protein starvation (Table 1). During this period, apparently, the growth processes proceeded very vigorously in the young animals, and the growth of the body considerably outstripped the growth of the ovary.

In the mature rats, depending on how long they had been fed the balanced diet, a progressive increase of both the absolute and relative weight of the ovaries was observed (Table 2).

In the immature rat group, the animals had still not reached sexual maturity by the 17th day of observation. After protein starvation, a few of the immature rats were left on the control diet for a 4-month period. After this period, not only did estrus appear, but the rats were capable of propagation.

In the mature rats fed the control diet after long protein starvation, the normal function of the ovaries was not restored immediately; the first estrus appeared on the 22nd-24th days. The inactive period between the first and the second estrus was longer than the ensuing ones, being 7 days in some rats, 12 in others. Then the normal sexual cycle was restored and repeated at the same intervals as in the animals kept on the control diet (see Figure)..



Sexual cycle of white rats under different conditions of protein feeding, a) control (diet with 19.5% protein); b) experiment (diet with 1.5% protein); c) restoration (diet with 19.5% protein); 1 — estrus; 2 — diestrus.

The results obtained from counting the structural components in the ovaries of the immature rats put on a balanced diet after protein starvation are given in Table 1. For the first 4 days of the balanced diet, there were no substantial changes in the number of ovarian structural components. The primordial follicles and follicles with destroyed oocytes were exceptions, as there were slightly more of them per section in the starved animals than in the rats on the control diet; the difference in the number of maturing and atretic follicles were not verified statistically.

The average number of maturing follicles did not change noticeably when the animals were kept on the control diet for a longer period (14-17 days). After 14-17 days of the balanced diet, the decreased number of atretic follicles and follicles with destroyed oocytes indicated that atresia was less intense. We were especially interested by the fact that the number of primordial follicles was greater in the ovaries of rats kept 14-17 days on the balanced diet than in the ovaries of rats kept only 4 days on this diet.

TABLE 1

Change in Ovary Weight and Structure in Immature Rats Fed the Control Diet after Protein Starvation

Animal group	No. of rats	Absolute wt. of ovaries in mg.	Relative wt. of ovaries in % $\pm$ m.	Average No. of structural components per section $\pm$ m				
				Primordial follicles	Maturing follicles	Corpora lutea	Follicles with destroyed oocytes	Atretic follicles
Rats kept on experimental diet for 64 days	6	4.5	14.1 $\pm$ 0.44	12.7 $\pm$ 0.42 (28.5%)	9.7 $\pm$ 1.07 (21.2%)	—	3.8 $\pm$ 0.09 (8.2%)	19.4 $\pm$ 1.32 (42.1%)
Rats kept on control diet for 4 days after starvation	5	7.5	16.3 $\pm$ 0.94	7.2 $\pm$ 1.20 (19.0%)	7.8 $\pm$ 0.79 (20.8%)	—	2.6 $\pm$ 0.32 (6.8%)	20.9 $\pm$ 2.60 (53.4%)
Rats kept on control diet for 14-17 days after starvation	5	8.4	12.9 $\pm$ 0.42	12.1 $\pm$ 2.13 (32.6%)	9.0 $\pm$ 0.59 (25.5%)	—	1.8 $\pm$ 0.23 (5.0%)	13.7 $\pm$ 2.34 (36.9%)

TABLE 2

Change in Ovary Weight and Structure in Mature Rats Fed the Control Diet after Protein Starvation

Animal group	No. of rats	Absolute wt. of ovaries in mg.	Relative wt. of ovaries in % $\pm$ m.	Average quantity of structural components per section $\pm$ m				
				Primordial follicles	Maturing follicles	Corpora lutea	Follicles with destroyed oocytes	Atretic follicles
Rats kept on experimental diet for 135 days	5	16.6	20.7 $\pm$ 1.28	7.7 $\pm$ 0.44 (17.9%)	2.8 $\pm$ 0.32 (6.4%)	6.4 $\pm$ 1.44 (14.1%)	4.2 $\pm$ 1.43 (8.7%)	23.6 $\pm$ 2.06 (52.9%)
Rats kept on control diet for 14 days after starvation	3	34.2	24.7 $\pm$ 3.10	15.4 $\pm$ 6.47 (34.1%)	5.4 $\pm$ 0.30 (12.4%)	8.6 $\pm$ 2.82 (20.1%)	1.0 $\pm$ 1.52 (2.1%)	13.6 $\pm$ 3.41 (31.3%)
Rats kept on control diet for 50 days after starvation	4	47.8	26.4 $\pm$ 1.50	5.4 $\pm$ 0.32 (15.4%)	10.1 $\pm$ 1.01 (28.5%)	9.3 $\pm$ 0.60 (26.3%)	0.9 $\pm$ 0.10 (2.4%)	9.7 $\pm$ 0.47 (27.4%)

New primordial follicles evidently form in the ovaries of animals put on the control diet after long protein starvation.

In the mature animals, on the 14th day after their transfer to the protein rich diet, no statistically verifiable differences in the average number of primordial follicles and corpora lutea could be discovered, as compared with that found in the ovaries of the starved rats (Table 2). The increased number of maturing follicles could be connected either with the decreased number of follicles undergoing regressive development or with the intensification of their differentiation from the primordial follicles. The decreased number of atretic follicles and particularly of the follicles with destroyed oocytes indicates the diminished intensity of atresia in the ovaries of the animals after 14 days of the balanced diet.

After 50 days of the balanced diet, there were considerably less primordial and atretic follicles, as well as less follicles with destroyed oocytes, in the rat ovaries than in those of the rats fed the protein deficient diet. The average amount of maturing follicles, however, increased (see Table 2). Consequently, after 50 days of the balanced diet, the differentiation process became intensified in the ovaries of the animals.

The change in the interrelation of structural components in the ovaries of the mature rats, after their transfer to the balanced diet, was about the same as in the immature rats. In both cases, atresia lessened, and the absolute number of primordial follicles increased. The latter was evidenced by the fact that the amount of primordial follicles per section was approximately the same as in the ovaries of the starved rats, whereas the weight and size of the ovaries, and consequently the number of ovarian sections, was considerably greater in the rats kept on the control diet.

On the basis of the research conducted, we concluded that the processes of growth and follicular differentiation are resumed in the ovaries of rats transferred to a balanced diet after long protein starvation: in mature rats, the follicles mature through ovulation, and new corpora lutea appear. The normal function and structure of the ovaries is restored.

#### SUMMARY

Sexually mature and immature rats kept for a long time on a diet deficient in protein (1.5% protein) were fed a control diet of 19.5% protein content. The functions, relative and absolute weight of the ovaries were restored. The microscopic structure of the organ was restored, likewise the growth processes and follicular differentiation were resumed. In comparison with the period of deficiency the number of oocytes markedly increased.

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\* In Russian.