QUANTITATIVE CHARACTERISTICS OF OOCYTES IN THE OVARIES OF WHITE RATS SUFFERING FROM ACUTE RADIATION SICKNESS

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White rats are widely used in radiobiological investigation of fertility and embryopathy. However, relatively little study has been done on the injury of the ovaries of these animals following total exposure to ionizing radiation in doses causing acute radiation sickness. The literature [1, 2, 3, 5, 6] inadequately elucidates the questions of differential radiosensitivity of the sex cells, development of the process as dependent upon the time after irradiation and the age of the animals, and the regenerative possibilities; the authors do not provide a detailed quantitative analysis of the obtained data.

Using local irradiation of the gonads in rats, a dependence was established between the number of oocytes that were retained, the dose of radiation, and the time after exposure [8, 10]. It was shown that the oocyte population decreases very quickly over the course of the first 18 h after irradiation; in this case, the most radiosensitive oocytes are those in the primordial follicles and the Graafian follicles.

The purpose of our work was to carry out a quantitative study of the oocytes in the ovaries of white rats suffering from acute radiation sickness.

EXPERIMENTAL METHOD

The investigation was carried out on 176 sexually mature white rats, weighing 150-180 grams, and 60 sexually immature white rats, weighing 80-90 grams. The control group consisted of 50 animals; the remaining animals were subjected to a single, total irradiation on the RUM-3 apparatus (force of 180 kv, current intensity of 10 ma, filters of 0.5 mm Cu and 1 mm Al, focal distance of 30 cm, radiation output of 37 r per minute), in a dose of 600 r. The rats were sacrificed at different intervals after the exposure, from 1 h to 6 mo. The ovaries were fixed in Zenker's solution with formalin, in a 10% solution of formalin, and with Shabadash's neutral fixatives, imbedded in paraffin, cut into serial sections with a thickness of 6 micra, and stained with hematoxylin-eosin, by the method of Fuelgen, the method of Shabadash-Hotchkiss, and according to the azan method. Using a binocular, stereoscopic microscope, we measured the maximum perimeters of the ovaries, and, according to the formula for the volume of an ellipsoid, we determined the volume of the gonads. In every fifth section we counted the number of retained and atretic primordial, growing and cavitated follicles transected in the region of the nucleolus. All the data were analyzed statistically, according to the method of Fisher-Student.

EXPERIMENTAL RESULTS

Ionizing radiation caused profound changes in the microscopic structure of the ovaries. Even in the first hours after irradiation the number of dividing follicular cells was reduced. Mitoses were absent from the granular zone of many growing and cavitated follicles. The structure of the oocyte nucleus was disrupted. The nucleoli were swollen. Clumps of DNP became coarser, and usually arranged themselves next to the nuclear membrane and nucleolus. Oxyphilia of the cytoplasm increased. The latter was often vacuolated in many oocytes. The processes of karyopyknosis increased.

A count of the sex cells showed that in the first 12 h after irradiation the content of follicles in the sexually

TABLE 1. Follicular Contents in the Ovaries of Sexually Mature Rats

Experimental conditions	Primordial		Growing		Cavitated		Number of ovaries
	retained	atretic	retained	atretic	retained	atretic	
A. Control	382 4,3[1]±0,8	74 0,7±0,3	129 1,5±0,4	38 0,4±0,2	118 1,3±0,2	37 0,4±0,09	6
B. 3 days after irradiation	107 1,2±0,3	312 3,2±0,9	72 0,7±0,2	125 1,2±0,1	68 0,7±0,1	87 0,7±0,2	7
C. 14 days after irradiation	162 1,3±0,2	93 0,8±0,2	65 0,5±0,1	76 0,6±0,1	55 0,4±0,06	101 0,9±0,2	10
D. 28 days after irradiation	34 0,4±0,1	51 0,6±0,1	15 0,17±0,07	54 0,7±0,09	15 0,17±0,05	39 0,4±0,1	6
E. 90 days after irradiation	56 0,6±0,1	36 0,4±0,04	14 0,15±0,04	38 0,4±0,09	18 0,2±0,09	30 0,2±0,04	6
F. 180 days after irradiation	20 0,2±0,04	5 0,04±0,01	5 0,05±0,02	18 0,2±0,02	6 0,06±0,02	28 0,3±0,1	7
Value of P Comparison between:							
A and B	0,02	0,0001	0,1	0,001	0,03	0,2	
B and C	0,8	0,009	0,3	0,009	0,05	0,6	
C and D	0,01	0,5	0,1	0,6	0,03	0,1	
A and C	0,001	0,7	0,02	0,3	0,0001	0,1	
A and D	0,001	0,7	0,02	0,1	0,0001	1,0	
B and D	0,05	0,02	0,03	0,04	0,003	0,2	
A and E	0,002	0,3	0,02	1,0	0,003	0,9	
A and F	0,001	0,05	0,008	0,1	0,0001	0,6	
B and E	0,1	0,02	0,04	0,002	0,01	0,03	
B and F	0,01	0,004	0,01	0,0001	0,0001	0,1	
C and E	0,03	0,2	0,1	0,3	0,1	0,03	
C and F	0,001	0,01	0,04	0,04	0,004	0,06	
D and E	0,3	0,3	0,9	0,6	0,5	0,1	
D and F	0,1	0,005	0,1	0,001	0,1	0,5	
E and F	0,01	0,002	0,2	0,07	0,08	0,4	

[1]Average number per section (M ± m).

mature rats differed little from the control. After 24 h, the number of retained primordial follicles decreased by a factor of more than 2, while among the growing and cavitated follicles we observed only a slight increase in artesia. By the 3rd day, a statistically significant decrease was observed not only in the primordial follicles, but also in the cavitated follicles ($P < 0.02$ and $P < 0.03$). The number of growing follicles also decreased during this period, but the difference from the control was not significant. This fact testifies to the great resistance of the oocytes in growing follicles as compared with the oocytes of primordial and cavitated follicles.

Following the exposure to ionizing radiation, the number of atretic follicles increased markedly. In this case, we noted a substantial difference between the number of atretic primordial and growing follicles, while the increase in cavitated atretic follicles was not statistically significant (Table 1). The latter fact may be due to an intensification in ovulation following irradiation [3].

In the course of the 4th-14th days, we did not note a subsequent significant reduction in the number of primordial follicles (Fig. 1). The individual retained follicles were normal in structure. The number of growing follicles for that period decreased to 0.5 ± 0.1 per single section, differing markedly from the figures for the control ($P < 0.02$). We observed a further decrease in the number of retained cavitated follicles. As a result of the resorption of atretic primordial and growing follicles, the number of cavitated follicles was reduced sharply, while the number of atretic ca cavitated follicles increased somewhat.

Studying the dynamics of the content of atretic follicles also showed that the basic mass of follicles dies in the

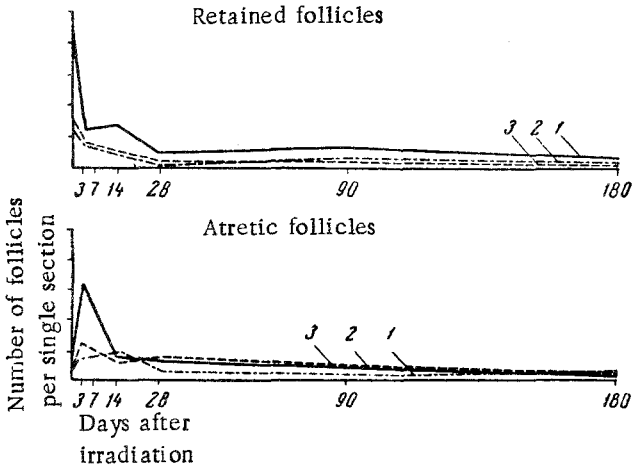

Fig. 1. The content of primordial (1), growing (2), and cavitated (3) follicles in the ovaries of sexually mature rats.

first days after irradiation. However, resorption of the atretic follicles occurs rather intensely, and is essentially finished by the 14th day.

At the end of the 4th week after exposure to ionizing radiation the sex cells had not completely disappeared. Despite marked disturbances in the morphology of the ovaries, they still contained individual follicles of normal structure. The single section retained up to 0.4 ± 0.1 primordial follicles, 0.17 ± 0.07 growing follicles, and 0.17 ± 0.05 cavitated follicles. The difference between these figures and the control was very significant ($P < 0.02$-0.0001). We observed a significant decrease in all types of follicles, as compared with their numbers in the 1st and 2nd weeks after irradiation.

At 3 months after the exposure, the gonads contained a small number of primordial, growing and cavitated follicles of normal structure. The increase in the number of primordial follicles to 0.6 ± 0.1, as compared with their number at 28 days after irradiation, was not statistically significant ($P > 0.3$). However, it should be taken into consideration that in the course of the 2nd and 3rd months the number of retained follicles ought to have decreased, but this not only did not happen, but, inversely, the number of primordial follicles even increased somewhat. This provides a basis for postulating that processes of postnatal oogenesis occur in the female gonads of irradiated rats.

At 6 months after the irradiation, we observed very few sex cells (see Table 1). However, there were individual primordial, growing and cavitated follicles of normal structure in almost every ovary. The decrease in the number of primordial follicles for the period from the 3rd to the 6th month was significant ($P < 0.01$). The reduction in the number of growing and cavitated follicles for this time period was not significant. We noted few atretic follicles.

There were significantly more primordial and growing follicles in the gonads of the control, sexually immature white rats than in the sexually mature animals ($P < 0.05$). After total exposure to ionizing radiation, analogous changes developed in the ovaries of the sexually immature white rats: mass death of the sex cells and follicular

TABLE 2. Follicular Content in the Ovaries of Sexually Immature Rats

Experimental conditions	Primordial		Growing		Cavitated		Number of ovaries
	retained	atretic	retained	atretic	retained	atretic	
A. Control	437 6,4[1]±0,7	20 0,3±0,09	192 2,8±0,06	28 0,4±0,009	85 1,2±0,3	46 0,6±0,01	6
B. 3 days after irradiation	35 0,4±0,09	77 1,0±0,5	32 0,4±0,1	57 0,8±0,2	36 0,4±0,1	73 1,0±0,3	6
C. 14 days after irradiation	53 0,7±0,1	9 0,12±0,08	40 0,5±0,1	34 0,5±0,1	36 0,5±0,07	61 0,8±0,1	6
D. 28 days after irradiation	19 0,3±0,1	10 0,2±0,1	14 0,2±0,02	30 0,46±0,2	5 0,1±0,05	35 0,6±0,05	3
Value of P Comparison bet.							
A and B	0,0000	0,1	0,0000	0,1	0,06	0,3	
B and C	0,1	0,1	0,3	0,3	0,5	0,6	
C and D	0,2	0,7	0,02	0,6	0,002	0,01	
A and C	0,0000	0,07	0,001	0,6	0,07	0,3	
A and D	0,0000	0,3	0,0000	1,0	0,01	1,0	
B and D	0,3	0,1	0,3	0,8	0,02	0,2	

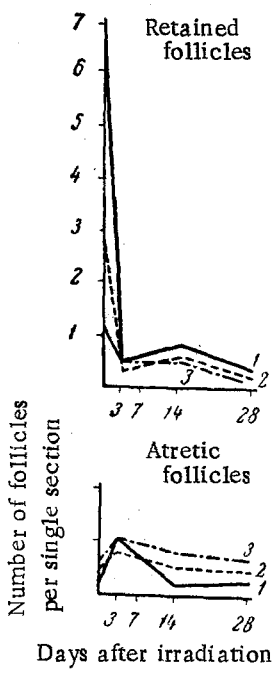

Fig. 2. Content of primordial (1), growing (2), and cavitated (3) follicles in the ovaries of sexually immature rats.

epithelium, hemorrhages, and disruption of the processes of cell division. However, radiation injury of the gonads in the sexaully immature animals was manifested more intensely (Table 2 and Fig. 2). In the first days, the number of normal follicles decreased more sharply, especially the primordial and growing types. In the sexually mature rats, the reduction in primordial follicles was 72%, while in the sexually immature – 94.1%; for the growing follicles, it was 54 and 89% respectively, and the cavitated follicles – 47 and 70% respectively.

Resorption of atretic follicles in the sexually immature rats apparently occurred much more rapidly, since the increase in their number was less marked.

The absence of yellow bodies must have definite importance among the possible reasons for greater radiosensitivity in the ovaries of the sexually immature animals, since it is known that these bodies somewhat decrease the degree of radiation injury to the follicular apparatus [4].

The size of the ovaries varied markedly in the different animals. In the first week after exposure, the mean volume of the gonads in the sexually mature rats did not change. After 2-4 weeks, it fell by 1.4-1.9 times. In the animals that were sacrificed 3 months after the irradiation, the mean volume of the gonads was significantly increased, due to the development of massive yellow bodies, cysts, and atypical growths, and exceeded the volume in the control rats by 1.6 times. At 6 months after the exposure, despite the development of massive ovarian tumors in individual animals, it was lower than in the control. The mean volume of the ovaries in the control, sexually immature animals exceeded the volume in the irradiated rats by 1.6 times. However, the change in the volume of the gonads did not reflect the dynamics of the sex cell content.

SUMMARY

A study was made of the content of persistent and atresic primordial growing and cavitary follicles in the ovaries of sexually mature and immature albino rats after general X-irradiation in a dose of 600 r. During the first days after the irradiation the main mass of the oocytes was seen to perish. The drop of the number of primordial follicles was more pronounced. No restoration of the sex cells occurred in the irradiated rats. However, up to 6 months after the action of ionizing radiation some primordial growing and cavitary follicles had a normal structure. The ovaries of immature rats were more radiosensitive. It has also been established that changes in the gonad volume did not reflect the dynamics of the sex cell content at various periods of acute radiation sickness.

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