RESTORATION OF OVARIAN STRUCTURE IN RATS FOLLOWING DISTURBANCES CAUSED BY ORTHOAMINOAZOTOLUOL

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The introduction of cancerogenic substances into an organism causes alterations in the weight and morphologic characteristics of the sex glands. However, data dealing with this question it scarce and extremely contradictory. It was discovered [7] that in rabbits that were smeared with tar the ovaries became heavier than in control animals. The increase in weight occurred as a result of an increase in the interstitial tissue. In the presence of a tar-induced tumor there takes place in rabbits a suppression of ovarian function [8], which is expressed as an inhibition of the development of follicles. A number of authors [5, 6] caused a lowering of the fertility of mice by smearing them with tar. A decrease in the function of the ovaries ("ovarian aging") was noted [4] in mice in which coal tar was applied to the skin. The effect of subcutaneous administration of 1, 2, 5, 6-dibenzanthracine was also studied [3]. This substance caused a lowering of ovarian function in mice which was manifested by a decrease in the number of large follicles and yellow bodies. However, the use of benzopyrene [2] did not cause similar changes in the mice ovaries. Upon smearing the mice with orthoaminoazotoluol over a period of 2-4 weeks [1] a decrease in the weight and function of the ovaries was noted; with more prolonged administration of orthoaminoazotoluol the weight of the ovaries increased more than 2 times in comparison with their weight in the control animals.

In those ovaries there was noted an increase in the proliferative processes.

The purpose of the present work involved the study of alterations in the function and structure of the rat's ovaries caused by prolonged administration of orthoaminoazotoluol and the possibility of restoration following these disturbances.

METHOD

Sexually mature rats, 198-244 g in weight, were used in the experiment. Along with its food, each experimental animal received 1 ml of a 6% solution of orthoaminoazotoluol daily in sunflower oil. Over the course of 7 months, the function of the sex glands was studied by means of the vaginal smear technique. The experimental animals, along with their corresponding control animals, were sacrificed at 1, 2, 3 $^1/_2$, and 7 months after the beginning of the experiment.

With the purpose of studying the restorative processes in the ovaries, one group of experimental rats, 10 in number, were kept under observation for an additional 5 months after the administration of orthoaminoazotoluol (for 12 months) had ceased. Then these animals were sacrificed. The left ovary was fixed in 80° alcohol for determination of the level of alkaline phosphatase by the method of Gomori [10] and of RNA by the method of Brachet [9]. The right ovary was fixed in Zenker's solution containing acetic acid for the study of the morphologic alterations and an appraisal of the structural components. For this purpose a series of sections were prepared, 7 μ in width; the preparations were stained with hematoxylin and eosin.

The structural components were counted in every 5th section of the ovary of the control and experimental rats after 3$^1/_2$ and 7 months of administration of orthoaminoazotoluol, as well as in the ovaries in the restoration period following the effect of orthoaminoazotoluol. In each group the structural components of the ovaries were counted in 7-10 rats.

RESULTS

In control animals with a normal rhythm of the sexual cycle and normal oogenesis a considerable amount of alkaline phosphatase and RNA was noted in the structural components and stroma of the ovaries. The cells of the germinal epithelium were very rich in RNA and alkaline phosphatase. Many enzymes were contained in the external, follicular, and ovarian layers of oocytes, which were in different stages of maturation. The cytoplasm and nucleoli of the follicular cells were also rich in RNA.

In the nuclei of the oocytes of primordial follicles the activity of alkaline phosphatase was high; dark-staining clumps of nuclear material were concentrated mainly next to the nuclear membrane. The nucleolus contained considerable amounts of enzyme and RNA. In the cytoplasm of the oocytes the amount of enzyme was less than in the nucleus, and it was here arranged in the form of thin granules; a small amount of RNA was also contained in the cytoplasm.

Along with the growth and maturation of the primordial follicles and their conversion, at first to young follicles and then to Graafian follicles, a lowering in the amount of RNA and alkaline phosphatase was observed in the cytoplasm of the oocyte. Infrequent clumps of RNA were localized around the nucleus of the oocytes and along the periphery of the cell, while fine granules of alkaline phosphatase were distributed throughout the cytoplasm. The nucleus of the oocyte in the young follicle contained a small amount of enzyme, distributed more frequently along the wall or to one side of the nucleolus. The nucleus of the oocyte in the Graafian follicle was free of enzyme. The nucleoli of the oocytes in the young follicles and the Graafian follicles contained much phosphatase and only a small amount of RNA.

In several of the oocytes in the Graafian follicles the nucleoli were low in alkaline phosphatase. Possibly this depended on the various stages of maturation or on some sort of functional peculiarities. In such follicles the capsules of the oocytes were rich in alkaline phosphatase, and the follicular fluid also contained a considerable amount of the enzyme but only traces of RNA.

The cytoplasm of the cells in the young yellow body contained a small amount of RNA, the clumps of which were arranged mainly around the nucleus. The nucleoli of the lutein cells contained a large amount of RNA and stained intensely with pyronine.

Alkaline phosphatase was found in the cytoplasm and nuclei of the lutein cells in the form of fine clumps. Yellow body cells were encountered with black nuclei, i.e., the activity of the enzyme here was very high; not a small number of these cells were found in which enzyme did not appear in the cytoplasm.

In the yellow body found in the stage of regression the cells became small, the amount of chromatin in the nuclei decreased, the nucleoli stained weakly with pyronine, and the RNA in the cytoplasm either completely disappeared or remained in an insignificant quantity. In both the cytoplasm and the nuclei of such a yellow body much enzyme was noted.

The alkaline phosphatase in the yellow bodies was not arranged in a single fashion; in the old yellow bodies it was in greater quantities in the nuclei, while in younger ones it was greater in the cytoplasm of the lutein cells (Fig. 1).

The administration of the cancerogenic substance over the course of 1-2 months did not cause essential changes in the function and structure of the ovaries.

After administration of orthoaminoazotoluol for $3^1/_2$ months a lowering of the body weight was observed in all the experimental animals. A reduction in the absolute and relative weight of the gonads was also noted. Thus, in the control group the weight of the two ovaries was 40.4 mg, while in the experimental group it was only

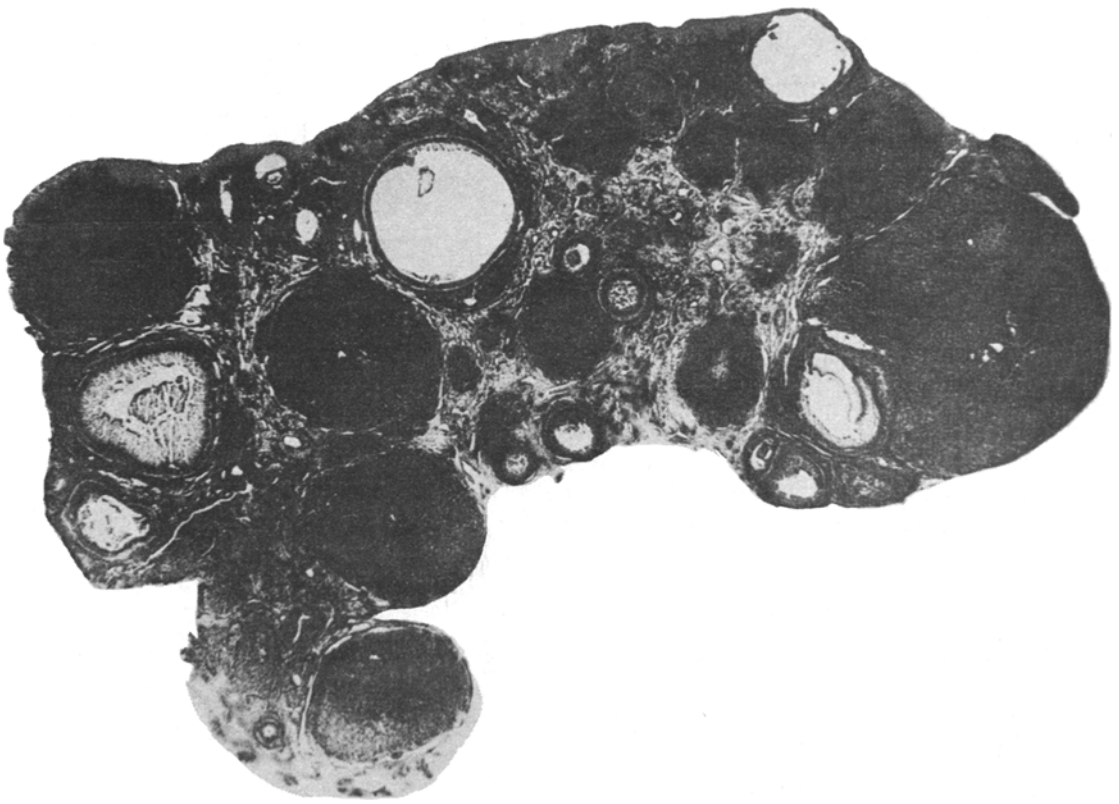

Fig. 1. Ovary of the control rat. Stained for alkaline phosphatase according to the method of Gomori. Ocular 5×, objective 4×.

Alterations in the Relationship of the Structural Components in the Ovaries of the Rat Following the Effects of Orthoaminoazotoluol Administration

Duration of the experiment (in months	Group of animals	Admin. duration of orthoaminoazotoluol (in months)	Weight of the body when sacrificed	Number of animals	Weight of the 2 ovaries		Average number of structural components of the ovary oer section					Total number of structural components
					absolute (in mg)	relative (in %	primordial follicles	mature follicles	yellow bodies	follicles containing disrupted oocytes	atretic follicles	
$3^1/_2$	Control		144	14	40,4	28,0	2,06	5,94	7,90	0,41	5,36	21,67
							(9,7%)	(27,2%)	(36,4%)	(1,8%)	(24,9%)	(100%)
	Experimental	$3^1/_2$	138	17	30,7	22,3	1,52	5,65	6,60	0.50	3,72	17,99
							(8,4%)	(31,7%)	(36,6%)	(2,8%)	(20,5%)	(100%)
7	Control		227	9	61,3	27,0	2,32	2,69	10,83	1,98	4,65	22,47
							(10,1%)	(12,0%)	(48,0%)	(8,7%)	(21,2%)	(100%)
	Experimental	7	198	10	41,7	21,0	0,43	3,15	9,55	1,14	5,89	20,16
							(1,9%)	(15,8%)	(47,4%)	(5,8%)	(29,1%)	(100%)
17	Control		244	7	37,6	15,4	1,28	4,28	1,65	0,66	3,71	11,58
							(11,2%)	(37,0%)	(14,7%)	(5,2%)	(31,9%)	(100%)
	Experimental	12	240	10	39,1	16,3	1,51	2,72	4,91	1,84	5,26	16,24
							(9,3%)	(16,7%)	(30,2%)	(11,1%)	(32,7%)	(100%)

30.7 mg. The difference is statistically valid. In this period the normal rhythm of the sexual cycle was disrupted in several of the experimental animals - the quiescent phase was prolonged. Utilizing histological investigations of these ovaries and a counting of the structural components, a decrease in the number of primordial and atretic follicles was discovered, which led to a lowering of the total number of structural components encountered per section. The processes of maturation and differentiation did not much distinguish themselves from those of the normal, which was pointed out by the count of the number of mature follicles and yellow bodies. The percent relationship of the structural components also failed to essentially change (see table).

The histochemical investigations did not demonstrate any changes in the ovaries of the experimental rats.

With the administration of the cancerogenic substance over the course of 7 months the same character of changes was preserved, but in several cases it was more clearly manifested. The body weight of the experimental animals was always lower than the body weight of the control rats. The absolute and relative weight of the ovaries in the experimental rats, despite the fact that they continued to grow, showed itself to be lower than in the control rats of that group: In the control the weight of the 2 ovaries was equal to 61.3 mg, in the experimental group 47.1 mg. The difference is statistically valid.

In these experimental intervals disturbance of ovarian function was observed in all the rats - the quiescent phase of the estral cycle was prolonged. At this time there was also encountered the most pronounced changes in the ovaries; not only was there observed a decrease in the total number of structural components seen in each section, but their percent relationship was altered as well. This appeared as a decrease in the number of primordial follicles and in an increase in the number of atretic follicles. The process of maturation and differentiation of the follicles was not disturbed, as is shown by the data on the number of mature follicles and yellow bodies (see table).

In the cells within the structural components of the ovaries of the experimental rats the character of the distribution of alkaline phosphatase and RNA was preserved, there being observed only a minimal lowering in the activity of the alkaline phosphatase and the amount of RNA, in the oocytes at various stages of their maturation, in the follicular epithelium cells, and in the yellow bodies. In isolated cases the enzyme did not appear in the follicular capsules, and only a small amount was preserved in the internal capsule of the follicle.

With prolonged administration of the orthoaminoazotoluol the ovarian capsules also lost a considerable amount of the enzyme. A reduction in the activity of alkaline phosphatase was observed in the germinative epithelium, in the nuclei of the ovarian stromal cells, and in the walls of the blood vessels (Fig. 2).

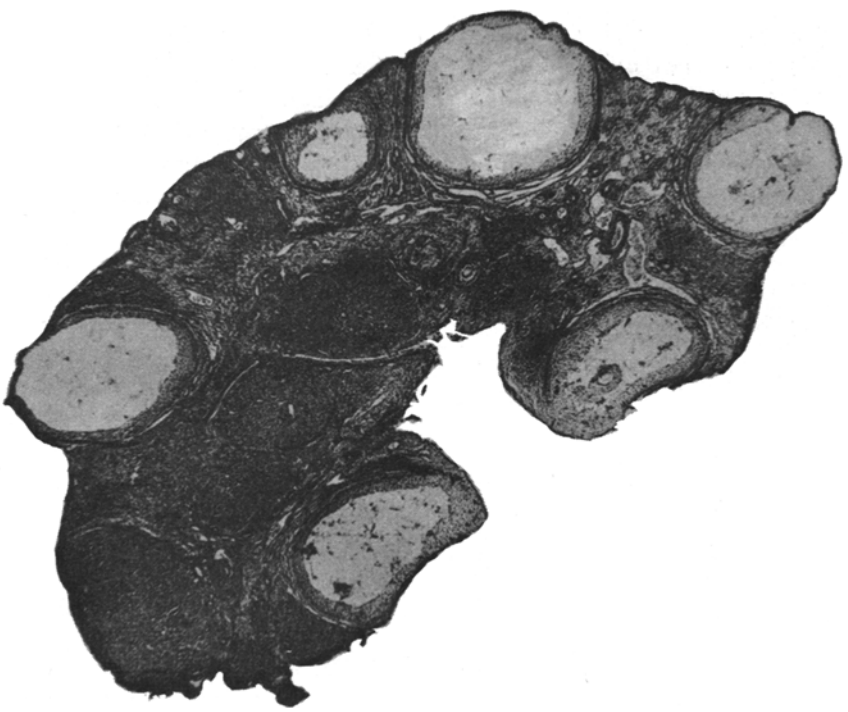

Fig. 2. Ovary of a rat after administration of orthoaminoazotoluol for 7 months. Stained for alkaline phosphatase according to the method of Gormori. Ocular 5×, objective 4×.

In the next group were included rats, which, over the course of 12 months, were fed orthoaminoazotoluol, following which the administration of the cancerogenic substance was stopped. In the ovaries of these of these animals, over the course of 5 months, the restorative processes proceeded. The absolute and relative weight of the ovaries increased. Thus, in the control group the 2 ovaries weighed 37.6 mg, while in the experimental group they weighed 39.1 mg.

The character of the ratio of structural components in the ovaries of the experimental rats in the period of restoration showed itself to be different from that in the ovaries of the control animals. These peculiarities manifested themselves by the presence of the considerable percentage of yellow bodies (in the control, 14.7%, while in the experimental group, 30.2%) and in a decrease in the percentage of maturing follicles (in the control, 37.0%, vs. only 16.7% in the experimental group). These numbers point out that in the ovaries of the control animals the alterations of aging, which usually occur at this time, were more strongly manifested, and the process of maturation and differentiation of the follicles was considerably inhibited. Atresia of the follicles occurred mainly in the intermediary stages of their development. At the same time, in the ovaries of the experimental animals during the period of restoration the process of maturation and differentiation of the follicles continued to remain intensive, and the total number of structural components in the ovaries of these rats showed itself to be greater than in the ovaries of the control animals (see table).

The increase in the number of primordial follicles in the ovaries of the rats in this group appeared as a result of their new formation. It is possible to conclude the latter on the basis of the fact that the number of primordial follicles encountered per section increased: in place of 0.43 it became 1.51. This conclusion is not altered even if one takes into consideration the fact that the weight and measurements of the ovaries of the experimental rats in this group were lower. However, the process of primordial follicle formation was not followed through by us and requires further investigation.

The activity of alkaline phosphatase and the amount of RNA in the young elements of the ovaries belonging to the experimental animals were restored to normal.

Thus, prolonged administration of orthoaminoazotoluol causes a disturbance in the function and structure of the ovaries of rats, which returns to normal after cessation of the administration of the cancerogenic substance.

SUMMARY

An orthoaminoazotoluol solution in sunflower oil in a daily dose of 0.001 ml was given to rats with food. Following $3\frac{1}{2}$ and 7 months of the cancerogenic substance administration there was noted a decrease in the size of these organs, associated with reduced activity of alkaline phosphatase and RNA content in the cells of the ovarian structural components. The disturbances noted in the rats' ovaries disappeared after the administration of orthoaminoazotoluol was discontinued. The weight of the ovaries and the process of follicular differentiation in them normalized. Restorative processes in the ovaries were observed in 5 months.

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