Changes in Mouse Ovaries After Prolonged Treatment with Cyclophosphamide[1] (34438)

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Cyclophosphamide ("Cytoxan," Endoxan") is an alkylating agent developed as an anticancer drug and recently used as an immunosuppressant. In a study of the use of cyclophosphamide in the treatment of rheumatoid arthritis, 6 of 33 women experienced cessation of menses after courses of 6 months or more (1). Here at Stanford, five young women being treated with this drug for systemic lupus erythematosus have suffered premature menopause and at autopsy of a similarily treated 13-year-old girl, there was complete absence of oocytes and follicles. For this reason, we have taken advantage of an ongoing experiment being carried out by one of us (L. J. Cole) to study the ovaries of mice given cyclophosphamide for a period of 1 year.

Methods. Ten female LAF_1 mice received 1.8 mg cyclophosphamide in 0.2 ml saline intraperitoneally once per week for 52 weeks starting at age 11 weeks. Nine female LAF_1 mice of the same age received 0.2 ml saline once per week for the same period. Five of the mice receiving cyclophosphamide and 4 of those receiving saline were killed at the end of the course of treatment. Each of the two remaining groups of five mice was then caged with two males. Three weeks later all the female mice were killed, and the number of pregnant animals and number of fetuses per pregnant mouse were determined. The ovaries from all mice were fixed, embedded in paraffin, sectioned at 5 μ by standard methods, and stained with hematoxylin and eosin. Similar sections were prepared of kidneys, spleen, thymus, and lymph nodes.

Two sections at different levels were prepared from each ovary, the sections were scanned, and the numbers of each of the following structures were counted: (1) primordial follicles (oocytes with a single layer of surrounding flat or cuboidal cells); (2) maturing follicles (follicles with two or more layers of lining cells); (3) corpora lutea; and (4) anovulatory rings (follicular-like structures of varied size with only a single layer of lining cells and no evident oocyte) (2). In each group, sections of one ovary included only portions of cysts, so totals of 19 ovaries were available for study from cyclophosphamide-treated mice and 17 ovaries from saline-treated mice.

Results. Grossly, all the animals appeared normal except that the drug-treated mice had gray hair. Microscopically no obvious lesions were found in the kidneys, spleen, thymus, or lymph nodes.

Examination of the sections of ovaries revealed that normal follicles and corpora lutea were rare in the cyclophosphamide-treated mice. The mean numbers of each structure per section are present in Table I. The ratio of variances for these data were high, so statistical analysis of the means was not performed. When the data were expressed as numbers of ovaries in which each structure was present or absent, χ^2 values using the Yates correction could be calculated, Table II. There were significant differences between the incidence of primordial follicles, maturing follicles, and corpora lutea in the ovaries of cy-

[1] Supported in part by the Bureau of Medicine and Surgery, U. S. Navy Department, the Hartford Foundation, and by Grant No. AI-08490 from the N.I.A.I.D., U.S.P.H.S.

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TABLE I. Mean Numbers of Each Follicular Structure Found per Section of Ovary.

	Treatment	
	Cyclophosphamide	Saline
Primordial follicle	0.05	3.2
Maturing follicle	0.35	5.4
Corpora lutea	0.05	1.7
Anovulatory rings	2.20	4.9

clophosphamide-treated compared to saline-treated mice (Fig. 1).

Aside from the presence of anovulatory rings in some of the ovaries from the drug-treated mice, microscopic examination revealed monotonous fields of undifferentiated stroma, a few small cystic or vascular spaces containing blood, and an apparently intact germinal epithelium (Fig. 2).

None of the five mice treated with cy-

TABLE II. Incidence of Presence or Absence of Types of Follicles in Ovaries from Cyclophosphamide- and Saline-Treated Mice.

	Treatment			
	Cyclophosphamide	Saline	χ^2	p
Primordial follicles				
Present	2	17	25	<0.01
Absent	17	0		
Maturing follicles				
Present	6	17	15	<0.01
Absent	13	0		
Corpora lutea				
Present	2	16	22	<0.01
Absent	17	1		
Anovulatory rings				
Present	16	16	0.17	<0.50
Absent	3	1		

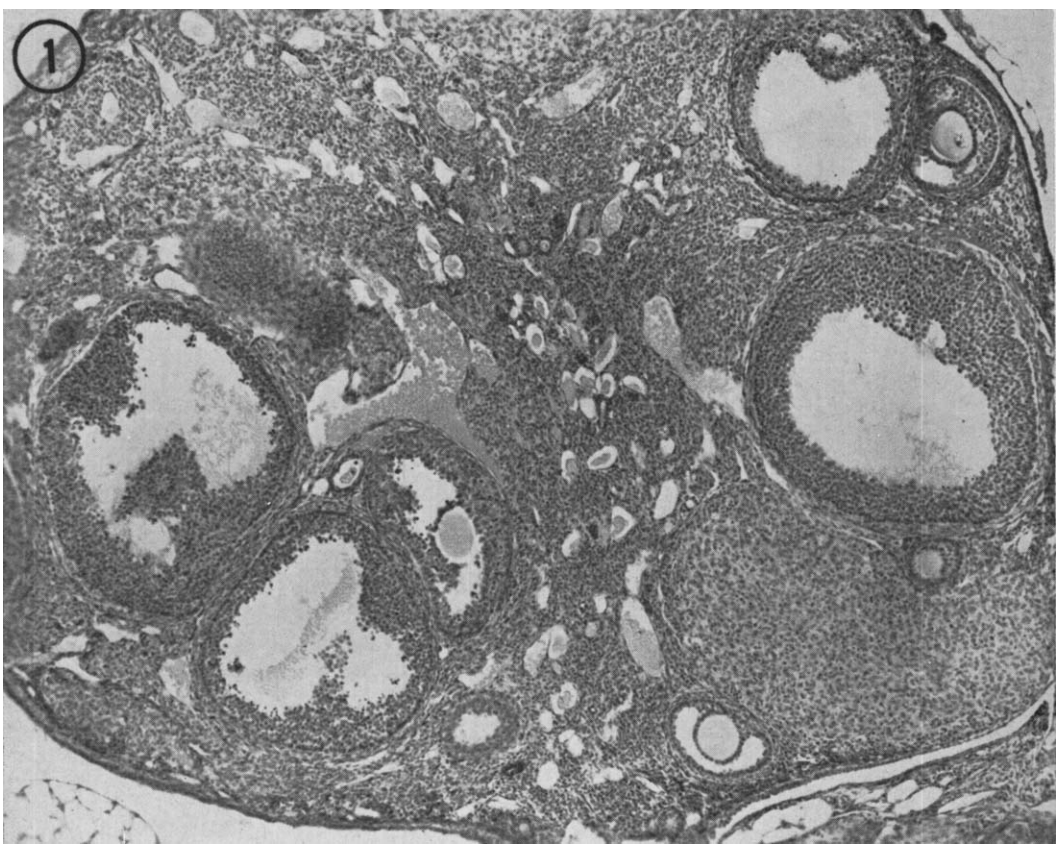

FIG. 1. Photomicrograph of a section of an ovary from a 14-month-old mouse treated with weekly injections of saline for 52 weeks. Maturing follicles at various stages of development and one corpus luteum are seen. ×75.

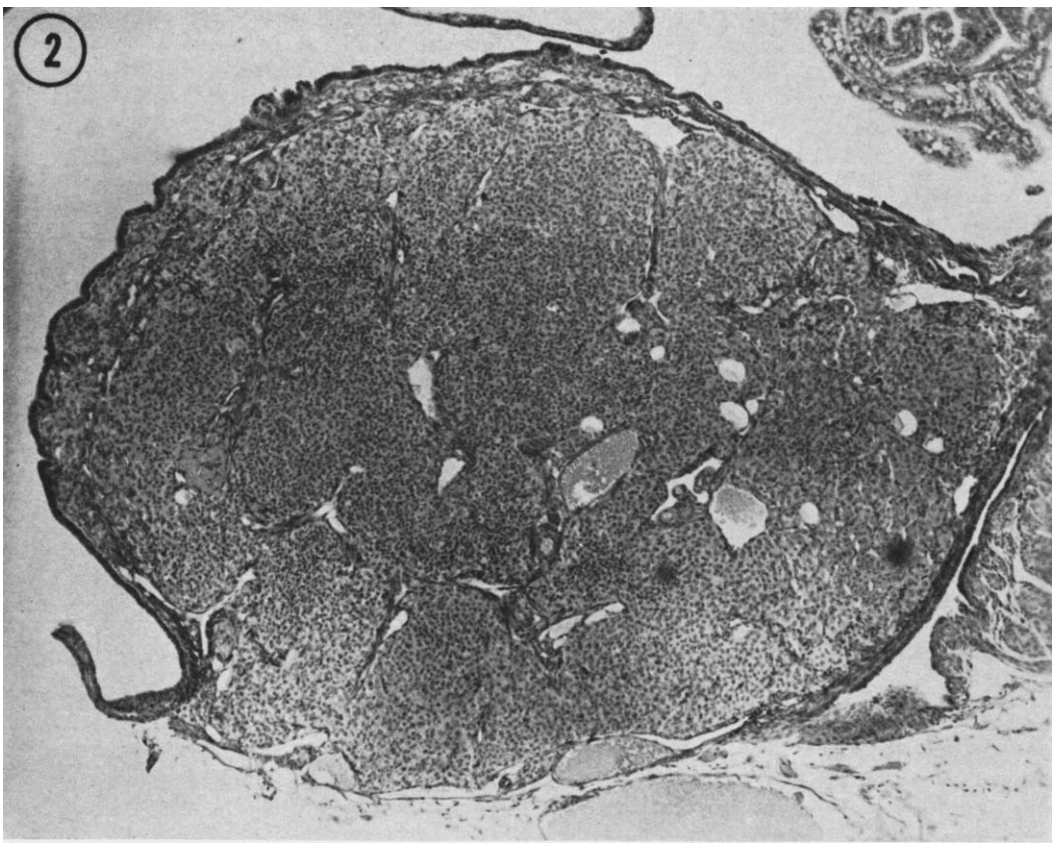

FIG. 2. Photomicrograph of a section of an ovary from a mouse treated with weekly injections of cyclophosphamide for 52 weeks. No follicles or corpora lutea are present, only an expanse of uniform-appearing stroma interrupted by vascular spaces. The dark border represents an intact layer of germinal epithelium. ×75.

clophosphamide became pregnant when exposed to males for 3 weeks. Three of the five mice treated with saline became pregnant with a total of 11 fetuses. This latter is consistent with earlier studies of the fertility of 14-month-old mice (3). The numbers involved are too small for statistical significance.

Discussion. These results indicate that prolonged treatment with cyclophosphamide interferes with the normal development of follicles and corpora lutea. Thus the fertility and normal hormonal cycles are presumably destroyed. The most obvious mechanism for such an effect would be interference with division of cells required for growth of the follicles. However, the decrease in numbers of primordial follicles implies that cyclophosphamide has a direct effect on the ovum for, as defined by us, this structure included all identifiable oocytes not in maturing follices. This is consistent with the irreversible ovarian failure which has been seen in humans treated with cyclophosphamide. The mechanism for this action on a presumably metabolically quiet cell is not clear. Since it appears that time is required for this effect, and since the doses used did not suppress bone marrow generation, the mechanism must be quite complex.

Summary. Cyclophosphamide given in sublethal doses for 1 year reduced the numbers of oocytes, follicles, and corpora lutea in mouse ovaries. The mechanism, unknown at present, is presumably related to that causing ovarian suppression in humans.

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Received Sept. 11, 1969. P.S.E.B.M., 1970, Vol. 133.