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Interactions of Triethylenethiophosphoramide, Estrogen and Gonadotropin On the Ovary of Hypophysectomized Immature Rat.* (26281)

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The dynamic morphologic changes occurring in the ovary during the normal ovarian cycle and under experimental conditions have been reviewed by Hisaw(1). Interference with these processes by cytotoxic agents which act most effectively on rapidly proliferating cells offers interesting avenues of study. The present report is concerned with disturbances provoked by triethylenethiophosphoramide (Thio-TEPA) on the ovary of the hypophysectomized immature rat and the influences of a pituitary gonadotropic preparation and diethylstilbestrol on such effects.

Methods. The experimental animals were immature rats of the Holtzman strain, hypophysectomized at age of 24 days. Injections were begun immediately after hypophysectomy and repeated daily for a total of 4 doses. At autopsy, 48 hours after final injection, the ovaries were quickly weighed, preserved in 10% buffered formalin and subsequently prepared for microscopic study by the standard Hematoxylin and Eosin technic. Ovarian weight was corrected to a final body weight of 100 g.

Thio-TEPA in a saline solution was administered intraperitoneally. The partially purified sheep anterior pituitary gonadotropin (Gonadophysin®) was administered subcutaneously in doses of .2 mg dissolved in .2 ml of saline. Diethylstilbestrol in doses of 1 mg dissolved in .1 ml of sesame oil was injected subcutaneously.

Results. Thio-TEPA administered alone to the hypophysectomized animals produced a slight but quantitative reduction in ovarian weight. The intense stimulation of increased ovarian weight by relatively large doses of estrogen or pituitary gonadotropin was more precipitously antagonized by Thio-TEPA. Maximal ovarian stimulation with the combination of gonadotropin and estrogen provided the most striking example of quantitative inhibition of ovarian growth produced by increasing doses of the antineoplastic agent (Table I).

Thio-TEPA alone in the smaller doses produced destruction of granulosa cells and follicular atresia, predominantly in the larger follicles. However, as the dose was increased this effect became more evident in smaller follicles. Destruction of primordial follicles

TABLE I. Effects of Thio-TEPA on Ovarian Weight in Hypophysectomized Immature Rat.

Treatment*			No. of		Mean ovarian wt, mg/100 g body wt ± S.E.
Thio-TEPA	DES	Gonadophysin	Animals	Survivors	
			10	10	18.07 ± .45
.1			3	3	15.65
.15			16	8	14.20 ± .97
	1		14	14	63.95 ± 2.20
.05	1		3	3	56.20
.1	1		18	11	44.20 ± 1.33
.15	1		23	12	39.20 ± 3.42
.2	1		4	1	25.70
		.2	5	5	41.68 ± 5.92
.1		.2	11	11	38.60 ± 3.63
.15		.2	14	11	31.80 ± 4.16
	1	.2	18	18	167.20 ±14.87
.1	1	.2	15	13	91.21 ± 4.70
.15	1	.2	19	13	68.20 ± 5.35
.2	1	.2	4	1	40.60

* Daily dose in mg administered for a total of 4 doses beginning immediately after hypophysectomy.

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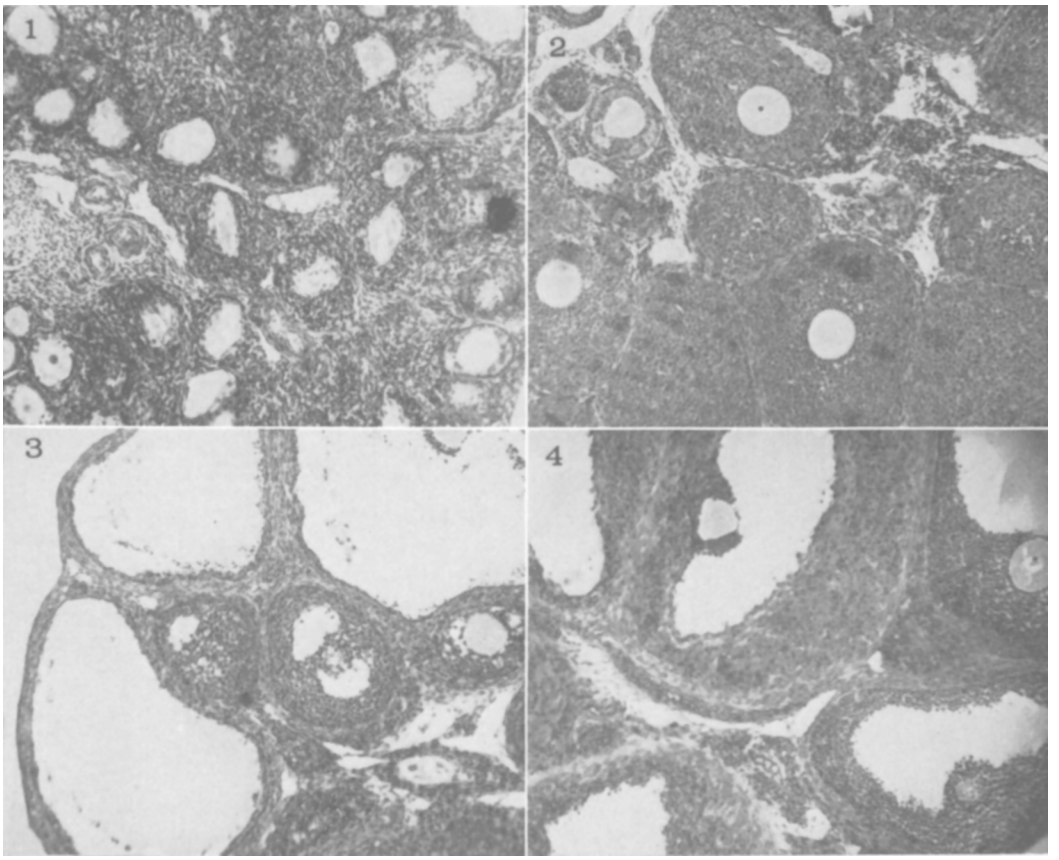

FIG. 1-4. Ovaries of hypophysectomized immature rats treated with 4 daily doses of indicated materials beginning immediately after hypophysectomy, with autopsy 48 hr after last inj. All magnifications × 60.

FIG. 1. .15 mg Thio-TEPA. Ovary is small. Follicular atresia and necrosis of ova in larger follicles. Primordial follicles unaffected.

FIG. 2. .15 mg Thio-TEPA + 1 mg DES. Follicular development to medium sized solid stage. Slight loosening of granulosa cells and early follicular atresia with an occasional necrotic ovum. Only sparse thecal and interstitial tissue.

FIG. 3. .15 mg Thio-TEPA + .2 mg gonadotropin. Widespread dissolution and pyknosis of granulosa cells, with atresia of follicles and cystic follicle formation. Most ova necrotic. Slight stimulation of thecal and interstitial tissue.

FIG. 4. .15 mg Thio-TEPA + .2 mg gonadotropin + 1 mg DES. Partial protection of granulosa cells and ova. Luteinization has occurred. Thecal and interstitial tissue are stimulated.

occurred only with the largest dose of Thio-TEPA. Necrosis of the ovum in the atretic follicle was generally apparent (Fig. 1).

Diethylstilbestrol (DES), in the large dose used, provided maximal development of ovarian follicles in the hypophysectomized immature rat as previously described(2). Such treatment with DES afforded substantial protection against the cytotoxic effects of Thio-TEPA on granulosa cells and ova (Fig. 2). An increased number of primordial follicles was present in the Thio-TEPA-DES ovaries in contrast to those treated with DES alone.

When gonadotropin in the dose employed in this study was administered alone to the hypophysectomized rat prominent vesicular follicles developed with occasional luteinization, as previously described(4). When Thio-TEPA in increasing doses was administered concurrently progressive atresia of developing follicles was observed, with destruction of granulosa cells and ova. Under these circumstances cystic follicles were formed (Fig. 3). Such ovaries resemble those produced

by small doses of pregnant mare serum gonadotropin in the hypophysectomized rat(3). Stimulation of thecal and interstitial cells by gonadotropin was only questionably impaired by Thio-TEPA and the observed narrowing of these zones was largely accountable to compression by encroachment of the cystic follicles. A rim of granulosa cells frequently persisted lining the membrana granulosa but no evidence of luteinization was seen in these cells.

When DES was added to gonadotropin-Thio-TEPA treatment, granulosa cells and ova were protected, and luteinization proceeded. Thio-TEPA appeared to increase the tendency for 'premature' luteinization, *i.e.*, luteinization of the follicle prior to ovulation, and to hasten atresia of the small solid follicles (Fig. 4).

Discussion. Cytotoxic effects of Thio-TEPA appeared to be directed primarily against rapidly proliferating granulosa cells. Destruction of ova and inhibition of cell growth in the theca interna and interstitial tissue were considered secondary to effects produced on granulosa cells. Thickening of a 'restricting' membrana granulosa under the influence of Thio-TEPA would also appear to be a secondary effect of the agent. Burkl and Kellner(5) have described thickening of the membrana granulosa as the preponderant disruptive effect of busulfan (Myleran®) on the ovary of the intact immature rat. Formation of cystic follicles without luteinization in the Thio-TEPA-gonadotropin treated animals may similarly represent an induced defect in membrana granulosa dissolution, offering speculation as to mechanisms by which cystic follicles are formed. However, such ovaries do not show development of theca interna and interstitial tissue to the degree that occurs following administration of gonadotropin alone, which might be attributed to crowding by the cystic follicles.

Protection of granulosa cells against Thio-TEPA by DES is of particular interest in view of intense stimulation of these cells by the estrogen. One would rather expect that such rapidly proliferating cells would be particularly susceptible to Thio-TEPA as has been reported with cells in tissue culture(6). It would be of interest to determine whether a similar protective effect of estrogen against Thio-TEPA applies to other cells.

Estrogen does not stimulate ovarian thecal or interstitial cell proliferation in the hypophysectomized rat(2). The present study demonstrates that DES facilitates luteinization in gonadotropin-Thio-TEPA treated hypophysectomized animals. The mechanism responsible for this effect is assumed to be a protective effect of DES on the follicle until follicular events are conducive to luteinization. It is of further interest that DES, in presence of gonadotropin stimulation and Thio-TEPA destruction, appears to promote direct luteinization of granulosa cells as manifested by premature luteinization.

Intense estrogen stimulation in the hypophysectomized rat slows formation of primordial follicles while producing an abundance of medium-sized solid follicles(2). The presence of primordial follicles in the Thio-TEPA-DES ovaries would indicate either persistence or increased production of primordial follicles by antagonism to development of larger follicles.

Summary. In the hypophysectomized weanling rat triethylenethiophosphoramide (Thio-TEPA) produced quantitative destruction of granulosa cells and ova. Stimulation of the ovary by pituitary gonadotropin was antagonized by Thio-TEPA, resulting in blocking of luteinization and formation of cystic follicles. Diethylstilbestrol partially protected against destructive effects of Thio-TEPA on the ovary allowing luteinization to proceed. Under all circumstances Thio-TEPA caused a quantitative reduction in ovarian weight over a period of 5 days.

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