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Effect of Estradiol on Immune Competence: In Vivo and In Vitro Studies

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The administration of a single dose of 2.5 μg of microcrystalline estradiol-17β from 1 day before and up until 3.5 days after the administration of 3×10^5 heat-killed *Escherichia coli* significantly increased numbers of splenic anti-*E. coli* antibody-producing cells in male mice sacrificed 4 days after receiving antigen. Administration early in the proliferative phase of antibody production, i.e., 1 day before or 1 day after the antigen, appeared to increase numbers of antibody-producing cells more than when it was administered at a later time. When given 2 days before the antigen or 2 h before sacrifice no effect was observed. Spleen cells harvested from male animals injected 3 days before with 5×10^6 heat-killed *E. coli* were incubated for 24 h in vitro with estradiol in concentrations ranging from 5 pg to 20 ng/ml. With concentrations of 500 pg to 5,000 pg/ml, significant increases in antibody-producing cells occurred, whereas at concentrations of 20 ng/ml some decrease was observed. The increase in antibody-producing cells was blocked by a mitotic inhibitor. Significant changes in numbers of antibody-producing cells were not observed after a 2-h incubation period. Uptake of titrated thymidine was increased in thymic and spleen cells incubated for 24 h with 500 pg of estradiol per ml; a concentration of 20 ng/ml slightly (but insignificantly) decreased uptake. Findings suggest that estradiol, in concentrations that approximate physiological serum levels in females, enhances mitosis of immunocompetent cells. This phenomenon may have bearing on the better immunological responsiveness of females than males.

Females are more resistant to infections and produce more antibody than males (3, 11, 18, 23, 24, 33, 35, 37). The mechanisms involved in these sex differences are largely unknown, although both genetic and hormonal factors have been considered important (1, 10, 12, 19, 21, 29, 31, 34, 35, 37). In previous studies in this laboratory, weanling and young adult female Swiss mice were found to have significantly greater numbers of splenic antibody-producing cells than males after oral and intraperitoneal challenges with *Escherichia coli* (16). In this outbred strain of mice, the larger number of antibody-producing cells appeared to result from an effect of female sex hormones, since ovariectomized females responded like males and the administration of small amounts of estradiol increased numbers of antibody-producing cells in male animals. The purpose of the studies reported herein was to determine when in the antibody-producing process estrogen exerts its effect and what mechanisms might be involved. Large amounts of natural and synthetic estrogens increase significantly the phagocytic clearance of particles from the blood (19, 29). It has been speculated that enhanced phagocytosis of antigens may be the reason for increased antibody production in animals that have received estrogen (19, 29). The findings of the present studies suggest, however, that small amounts of estradiol may have an effect on the proliferation of immunocompetent cells independent of phagocytosis. In studies in which splenic antibody-producing cells were enumerated by an agar plaque technique, numbers of plaque-forming cells were increased when estrogen was administered to animals as late as 3.5 days days after the antigen. In vitro studies further suggested that physiological amounts of estrogen may affect the proliferation of immunocompetent cells at a time long after the antigen has been phagocytosed.

MATERIALS AND METHODS

Test animals. Litters of male Swiss Webster weanling mice, 12 to 15 g, were acquired from a local breeder. Each litter was divided into two or three equal parts, and mice from each individual

litter were distributed equally in control and test groups. One control group and one or two experimental groups, each consisting of 20 to 30 mice, were studied simultaneously. In most experiments mice were tested when they were 3 to 4 weeks old. In each experiment a total of 40 to 60 mice was studied, and each experiment was repeated two to five times. Spleen cells from adult male mice 2 to 5 months old were used for in vitro studies.

Antigen. Heat-killed *E. coli* 0127 K63 (B8) for injection and soluble O antigen for modification of sheep erythrocytes were prepared as described previously (16).

Estradiol. A single dose of an aqueous microcrystalline suspension of estradiol (Progynon; Schering Corp.), 2.5 μg in 0.1 ml of saline, was administered intramuscularly to the thigh at various times prior to or after the administration of antigen. Stock suspensions of 0.25 or 1 mg/ml were diluted in saline prior to use. Levels of estradiol in serum were measured in the laboratory of Frederic Kenny using a radioimmunoassay (2). Blood from 10 male mice injected with 2.5 μg of estradiol was collected 1 h after injection by section of axillary vessels. In one experiment serum from these estradiol-treated mice or normal male mice was added to the agar at the time the cells were plated (0.2 ml/ml of agar). Solutions of estradiol (1 mg/ml) were prepared in absolute ethanol from stock powder. Appropriate dilutions were made in saline so that they could be added to media at the time of plating or to tissue culture dishes in 0.04- to 0.1-ml amounts to give final concentrations of 5 pg, 50 pg, 500 pg, 5 ng, or 20 ng per ml of media.

Immune response. (i) In vivo studies. Anti-*E. coli* antibody-producing cells were enumerated 4 days after the intraperitoneal injection of 3×10^5 heat-killed *E. coli* by the Jerne-Norden technique as in prior studies (16). All cell suspensions were plated with unmodified erythrocytes as well as with those modified with *E. coli* antigen. Numbers of plaques on plates prepared with the unmodified erythrocytes were subtracted from those prepared with modified cells. Initially agar (Bacto-agar, Difco) with diethylaminoethyldextran (0.5 mg/ml) was used for plating cells (13); in later experiments agarose was used as described by Kaliss (15). Although total counts of anti-*E.coli* hemolytic plaques were 10 to 1,000 times higher when agarose was used as the suspending medium, the counts of test and control groups relative to one another within a given experiment were the same.

(ii) In vitro studies. Suspensions of spleen cells were prepared 3 days after male mice had been injected intraperitoneally with 5×10^6 heat-killed *E. coli*. Spleen cells were suspended in 10 ml of Spinner's medium. Cells were washed once and resuspended in RPMI 1640 medium buffered with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (30) to which was added 25 U of polymixin B per ml, 100 U of penicillin per ml, and 100 μg of kanamycin per ml. Each spleen suspension containing 10^8 to 3×10^8 cells/ml was placed in two 5-ml amounts in petri dishes (Falcon Contur dish, 3 by 1.5 cm). Estradiol was added to one 5-ml aliquot of the suspension, and both estrogen-treated and control suspensions of the same spleens were incubated in 5% CO_2 and air. In a few experiments a mitotic inhibitor, colcemid (40 μg/100 ml), was added to the medium of both test and control suspensions prior to incubation. After 2 or 24 h of incubation, spleen cells were washed, resuspended in 2.5 ml of Eagle medium, and iced until they were plated for enumeration of anti-*E. coli* plaque-forming cells.

Before plating, suspensions were randomly numbered and coded so that plaques could be counted without knowledge of prior treatment of the suspension. Viability was determined at the time of plating by 0.1% trypan blue exclusion, and total cells in each suspension were determined by use of a Neubauer chamber. In final analysis the number of plaques per 10^8 viable cells in each suspension was calculated, and a ratio of number of plaque-forming cells in the hormone-treated suspension to that in the control suspension was determined.

As a measure of deoxyribonucleic acid synthesis, uptake of tritiated thymidine by spleen or thymus cells was determined as described by Vischer (30). Mice were injected 3 days before sacrifice with 5×10^6 heat-killed *E. coli*. Cells were suspended in Spinner's medium, washed once, and resuspended at a concentration of 10^6 cells/ml in RPMI 1640 medium with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid buffer, to which was added 25 U of polymixin per ml, 100 U of penicillin per ml, and 100 μg of kanamycin per ml. Duplicate suspensions were distributed in 1-ml amounts in petri dishes. Estradiol was added to one dish and 1 μCi of tritiated thymidine (specific activity, 15 μCi/mg) was added to both dishes. After 18 to 24 h of incubation in 5% CO_2 and air, counts per minute of trichloroacetic acid-precipitable protein for each suspension were determined in a Packard Tri-Carb liquid scintillation counter.

Statistical analysis. Data were analyzed by nonparametric methods, using chi-square and sign tests. A P value of <0.05 was considered significant.

RESULTS

Levels of total estrogen achieved in serum after one injection of 2.5 μg of crystalline estradiol-17β as determined on pooled samples from 10 mice at various times after injection are shown in Fig. 1. The level rose to 375 pg/ml at 1 h, rapidly fell to 35 pg/ml by 12 h, and was at 23.5 pg/ml at 72 h, a level which approximated the serum levels of most control weanling males. In a limited number of determinations of total serum estrogen for normal male mice of 3 to 6 weeks of age, levels have ranged from 20 to 40 pg/ml; levels for females of the same age, simultaneously determined, have been 10 to 30 pg/ml higher. Frequency distributions of numbers of plaque-forming cells in representative experiments in which estradiol was given at various times prior to and after the injection of antigen are shown in Fig. 2. Responses

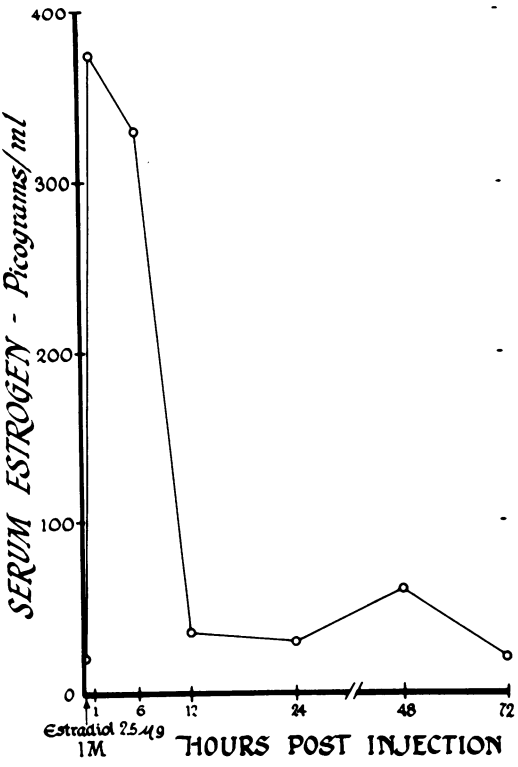

FIG. 1. *Concentration of total estrogen in serum of male weanling mice after an injection of 2.5 μg of crystalline estradiol-17β intramuscularly. Each point represents concentration in pool of serum from 10 mice.*

of animals that received estradiol have been compared to those of saline-injected controls. Estradiol given 2 days before the antigen had no effect, but administration of estradiol 1 day before and up to 3.5 days after the antigen (12 h prior to sacrifice) did increase numbers of anti-*E. coli* antibody-producing cells. Estrogen given 2 h before sacrifice had no effect. In each instance responses of animals in test and control groups were ranked together and distributions were analyzed by comparison of numbers of each group in quarters, thirds, and halves of the rank order. Table 1 shows the results of five experiments in which animals receiving estradiol 1 and 3 days after the antigen and controls receiving saline were tested simultaneously. Greater numbers of responses of animals receiving estradiol 1 or 3 days after the antigen were in the upper and middle thirds of the rank order than those of the saline-injected controls ($P < 0.025$ and < 0.01, respectively). Similarly, in experiments in which the responses of 40 mice receiving estradiol 12 h before sacrifice were ranked with those of the same number of saline-injected controls, significantly greater numbers of those of estradiol-treated animals were in the top quarter of the rank order (and fewer in the lower three-quarters) ($P < 0.05$). Mean plaque counts for test and control mice within individual litters were compared. In those test situations for which more than 18 litters had been studied the results were analyzed statistically. These comparisons are shown in Table 2. By use of this form of comparison, statistically significant differences were found between means of test and control animals only when the estradiol was given early (1 day before and 1 day after antigen).

In vitro studies. The question arose as to whether estrogen directly influenced the functioning of antibody-producing tissues or whether the effects observed were due to some other humoral factor, the production of which was stimulated by estradiol. The addition of estradiol, 50 to 500 pg/ml, to the agar media at the time of plating had no effect on numbers of antibody-producing cells. Similarly, the addition to the agar of serum from animals treated with estradiol produced no noticeable change in the numbers of antibody-producing cells. Counts of plaques for the same suspension plated with normal mouse serum and with serum from estradiol-treated animals were not different. Therefore, further studies were performed using an in vitro cell culture system. Spleen cells from mice that received injections of antigen 3 days before were incubated for 24 h with different concentrations of estradiol ranging from 5 pg/ml to 20 ng/ml. Results are shown in Tables 3 and 4 and Fig. 3. Table 3 shows the number of suspensions treated and mean ratios of plaque-forming cells in the estradiol-treated suspensions over those in the controls. The mean of these individual ratios is shown for suspensions with ratios greater and less than one. In Table 4 ranges and geometric means for plaque-forming cells per 10^8 viable spleen cells for hormone-treated and control portions of the suspensions tested are shown. In Fig. 3a a ratio (designated as E/C) has been calculated by using total plaques from all estradiol-treated suspensions over total for all of the controls. Figure 3b shows percentage of the total suspensions for which the ratios of individual suspensions were greater than 1. Suspensions treated with 500 pg/ml to 5,000 ng/ml had significantly increased numbers of antibody-producing cells. Suspensions treated with 50 pg/ml also showed some increase, but the proportion of suspensions with an increase was not significant. At a concentration of 20 ng/ml some reduction in numbers of antibody-producing cells was observed. Numbers of antibody-pro-

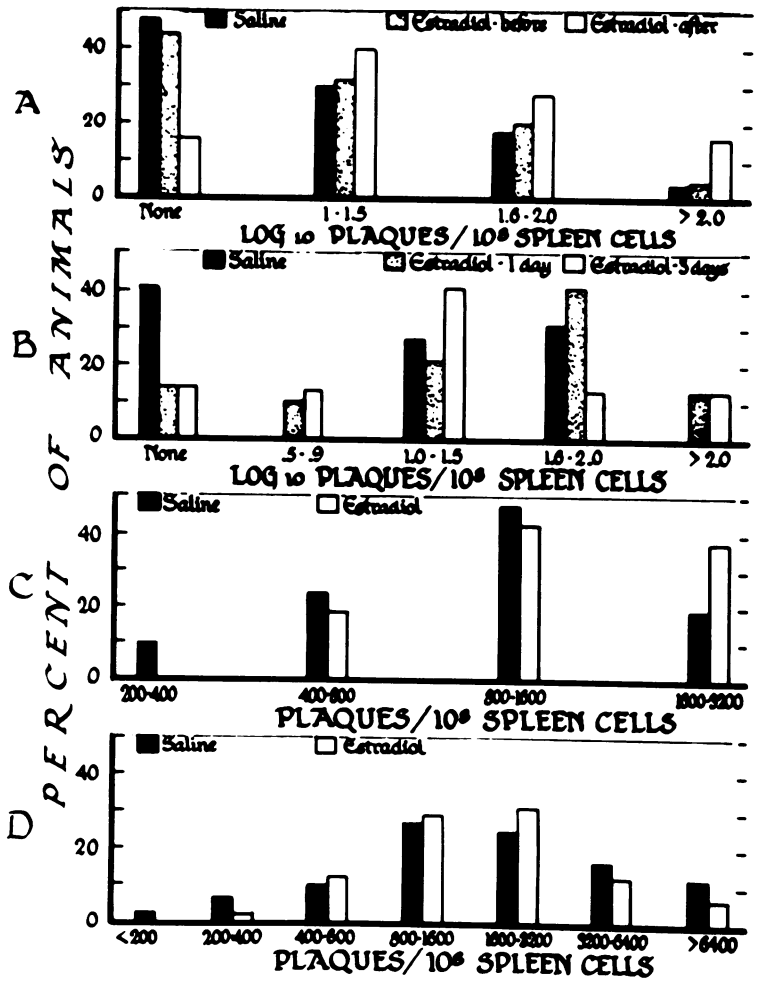

FIG. 2. *Effect of 2.5 μg of estradiol administered intramuscularly to male weanling mice at various times prior to or after the injection of 3 × 10⁵ heat-killed E. coli. Frequency distributions show anti-E. coli plaques/10⁸ spleen cells for estradiol-treated animals and equal numbers of saline-injected controls. All were sacrificed 4 days after receiving antigen. (a) Estradiol given 2 days before and 2 days after the antigen; (b) estradiol given 1 day and 3 days after the antigen; (c) estradiol given 3.5 days after the antigen (12 h bfore sacrifice); (d) estradiol given 2 h before sacrifice. Agar (Bacto-agar, Difco) was used for plating cells in (a) and (b), agarose was used in (c) and (d).*

ducing cells were reduced slightly in 75% of these suspensions.

To determine whether brief or longer exposure to the estradiol was required to obtain the enhancing and suppressing effects, suspensions were incubated for 2 h, as well as for 24 h, with 500 pg or 20 ng of estradiol per ml. To determine if the effects of estradiol might be the result of stimulation of mitosis, in some experiments colcemid, a mitotic inhibitor, was added to the medium of test and control suspensions. Results are shown in Tables 3 and 4 and Fig. 4. Although a significant proportion of the suspensions treated with 500 pg/ml or 20 ng/ml for 24 h, respectively, produced increased or decreased numbers of plaques compared to their controls, incubation with these concentrations of estradiol for 2 h did not result in a significant change. When colcemid was added to the media for a 24-h incubation period, only 13 of 34 suspensions treated with estradiol produced more plaques than their controls, indicating that the colcemid blocked the effect of estradiol.

To obtain further evidence that estradiol alters rates of division in lymphoid cells, the uptakes of tritiated thymidine by thymus and spleen cells treated for 24 h with 500 pg and 20 ng of estradiol per ml were compared with those

TABLE 1. *Effect of estradiol (2.5 μg) administered 1 or 3 days after 3 × 10^5 heat-killed E. coli 0127*

Experimental group (no. tested)	Rank order of no. of anti-*E. coli* plaque-forming cells/10^8 spleen cells[a] in male mice		
	Upper third	Middle third	Lower third
Saline-injected controls (65)	15 (23)[b]	19 (29)	31 (47)
Estradiol of 1 day after antigen (64)	27 (42)	20 (31)	17 (27)[c]
Estradiol 3 days after antigen (60)	21 (35)	25 (42)	14 (23)[d]

[a] Summary of five experiments.
[b] Numbers in parentheses are percentages.
[c] $P < 0.25$.
[d] $P < 0.01$.

of the same suspensions that were not treated with hormone. Findings are shown in Tables 5 and 6. Table 5 shows mean ratios of counts per minute in the hormone-treated portions over those for the control portions of the individual suspensions tested and numbers of suspensions for which these ratios were greater and less

TABLE 2. *Proportion of litters for which mean response of estradiol-treated animals was greater than that of their littermate controls*

Parameter	Time of administration of estradiol in relation to antigen			
	1 day before	1 day after	3–3.5 days after	4 days after
Proportion of litters	14/19	46/72	28/53	28/56
Percentage	73	63	52	50
P	0.032	0.012	>0.2	>0.2

TABLE 3. *Effect of estradiol on numbers of anti-E. coli plaque-forming cells in spleen. In vitro studies*

Concn of estradiol (pg/ml)	Incubation period (h)	Total suspensions tested	No. of E > C[a] (%)	Mean E/C[b]	No. of E < C (%)[c]	Mean E/C[b]	*P*
5	24	17	9 (52)	2.50	8 (48)	0.423	>0.2
50	24	57	33 (58)	1.99	24 (42)	0.588	0 145
500	24	50	33 (66)	2.53	17 (34)	0.570	0.017
5,000	24	52	33 (63)	2.93	19 (37)	0.538	0.035
20,000	24	32	8 (25)	3.28	24 (75)	0.557	0.004
500	2	31	18 (58)	1.75	13 (42)	0.682	>0.2
20,000	2	39	16 (41)	2.76	23 (59)	0.569	>0.2
500 (with colcemid)	24	34	13 (38)	2.12	21 (62)	0.556	0.115

[a] E > C, Suspensions tested that estradiol-treated portion produced more plaques per 10^8 viable cells than control portion.
[b] E/C, Ratio of number of plaque-forming cells in estradiol-treated suspension to that in control. Mean is average of ratios of individual suspensions.
[c] E < C, Suspensions tested that control portion produced more plaques per 10^8 viable cells than estradiol-treated portion.

TABLE 4. *Effect of estradiol on numbers of anti-E. coli plaque-forming cells in spleen. In vitro studies*

Concn of estradiol (pg/ml)	Incubation period (h)	Total suspensions tested	Ranges and geometric means of plaque-forming cells per 10^8 viable spleen cells				% Change of mean
			Suspensions without hormone		Suspensions with hormone		
			Range	Mean	Range	Mean	
5	24	17	69–8,333	835	134–10,096	769	−8
50	24	57	714–94,139	8,090	305–99,495	8,830	+9
500	24	50	44–89,876	3,312	63–106,742	5,395	+63
5,000	24	52	194–146,875	2,377	353–158,076	3,476	+46
20,000	24	32	349–108,333	2,812	61–136,257	2,108	−25
500	2	31	42–626,000	9,463	102–454,286	10,420	+10
20,000	2	39	226–189,707	6,502	114–118,889	6,607	−2
500 (with colcemid)	24	34	83–636,474	10,000	204–489,394	9,016	−10

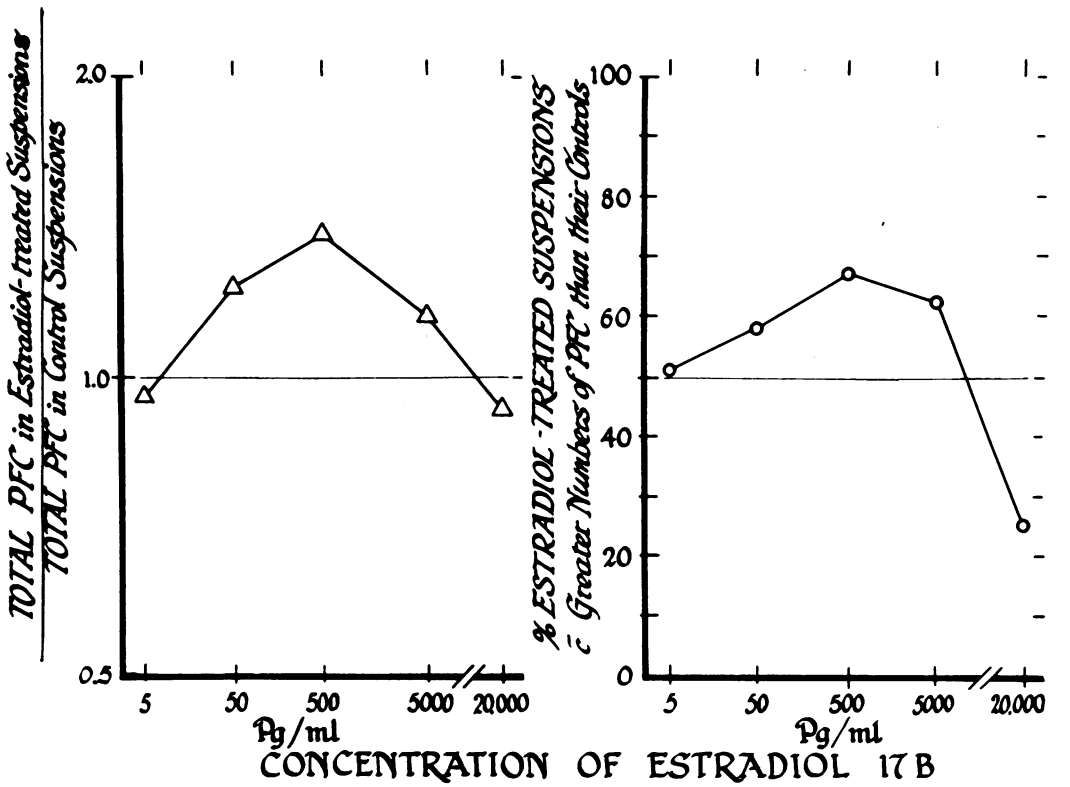

FIG. 3. *Numbers of plaque-forming cells (PFC) in spleen suspensions incubated in vitro for 24 h with various concentrations of estradiol. Each spleen suspension from an animal immunized 3 days before was incubated with and without estradiol. (a) Ratio of total plaques of all estradiol-treated suspensions over total plaques of the controls for each concentration of estradiol tested. (b) Percentage of individual suspensions tested for each concentration of estradiol for which number of plaque-forming cells in estradiol-treated portion was greater than number in control portion.*

than one. Table 6 gives ranges and geometric means of counts per minute per 10^6 cells for each group of determinations. Slightly greater counts per minute were observed for the majority of spleen and thymus suspensions treated with the small dose of estradiol, whereas larger amounts slightly but insignificantly suppressed counts.

DISCUSSION

Natural and synthetic estrogens significantly stimulate reticuloendothelial function, in short term by increasing the numbers of phagocytic cells that take up particles (5) and over a long term by increasing total numbers of phagocytic cells in reticuloendothelial tissues (29). After a single subcutaneous dose of estrogen in oil, an increase in phagocytic activity occurs within 12 h, reaches a maximum within 2 to 3 days, and then declines to original levels within 8 to 12 days. (29) Large doses of hormone have been used in most of these studies of phagocytosis, but physiological levels in females may also have some effect since reticuloendothelial function has been found to change during the estrus cycle, increased function paralleling periods of increased estrogen production (19). In studies of phagocytic function in estrogen-treated animals, reported by Nicol and his colleagues, increases in serum immunoglobulin were found to occur and were considered secondary to the phagocytic enhancement (19). The effects of estrogens on serum immunoglobulin or antibody levels as reported by others, however, have been variable, some reporting an increase (4, 23, 28, 32, 34), others a depression (3, 22, 25, 27), and some no change (6).

The results of the present study suggest that the administration to male animals of a single small dose of estradiol, which produces a peak approximately 10 times physiological levels and then levels slightly above the physiological range over a period of several days, increases numbers of antibody-producing cells. The effect was observed when the estradiol was

given 1 day before the antigen and up to 3.5 days after the antigen. Administration of the estradiol 2 days before the antigen, which resulted in serum levels slightly above physiological range at the time the antigen was injected but in physiological levels during the proliferative phase of antibody production, appeared to have no effect. These results suggest that estradiol in this amount and/or form is insufficient to produce prolonged enhancement of reticuloendothelial function as described above and increase antibody production via enhancement of phagocytic mechanisms. The in vitro studies of antibody-producing cells harvested late in the proliferative phase of antibody production further suggest that the phenomenon we have observed is independent of phagocyte function and that estradiol directly affects lymphoid cells in the spleen. This increase in antibody-producing cells was observed after a 1-day incubation period with estradiol and could be blocked by a mitotic inhibitor. These findings suggest that small amounts of estradiol stimulate mitosis of immunocompetent cells rather than accelerating differentiation of these cells. Another possibility is that estradiol activates T lymphocytes, which may play a role in regulating the division of immunocompetent cells. These possibilities are further suggested by studies that show that these small amounts of estradiol increase the uptake of tritiated thymidine in both thymus and spleen.

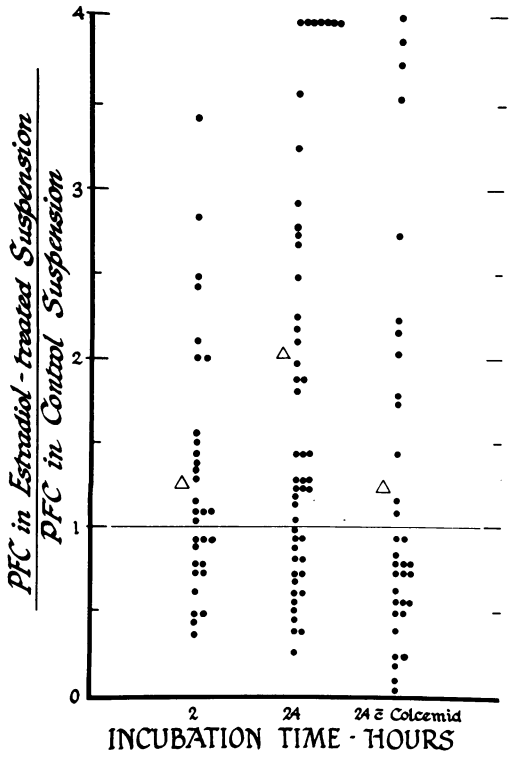

FIG. 4. *Effect of incubation of spleen cells with 500 pg of estradiol per ml for 2 and 24 h and 24 h with colcemid. Each dot represents a ratio for the number of plaque-forming cells in the estradiol treated portion of each suspension over that in the control portion. Triangles represent the means of the individual ratios.*

It is well known that estradiol stimulates nucleic acid synthesis and cell division in reproductive system tissues. This effect occurs as a result of the shortening of the S phase or phase of deoxyribonucleic acid synthesis of the cell cycle (7, 8). It is now known that estrogen may affect rapidly dividing nontarget tissues in a similar way, although to a less degree (8, 9). Epifinova showed that during division of corneal epithelial cells the interphase of the cell cycle is decreased 1.5 times in animals treated with estrogen (7, 8). Others have shown similar effects of estrogen on intestinal and buccal epithelial tissues (9, 14). Estrogen may have a similar effect on lymphoid tissues, since histologically the number of mitoses in these tissues increases in animals treated with estrogen (17).

The results of the in vitro studies suggest

TABLE 5. *Effect of estradiol on uptake of tritiated thymidine by thymus and spleen*[a]

Concn (per ml)	Type of cells	No. of suspensions tested	No. of E> C[b] (%)	Mean E/C[c]	No. of E< C[d] (%)	Mean E/C[c]	P
500 pg	Spleen	59	40 (68)	1.94	19 (32)	0.744	0.004
500 pg	Thymus	15	12 (80)	1.47	3 (20)	0.675	0.018
20 ng	Spleen	64	34 (53)	1.08	30 (47)	0.869	>0.2
20 ng	Thymus	67	29 (43)	1.44	38 (57)	0.733	0.164

[a] Counts per minute of 10^6 cells determined after 24 h of incubation with 1 μCi of tritiated thymidine.
[b] E > C, Suspensions treated with estradiol that had counts per minute greater than those of control.
[c] E/C = counts per minute of estradiol-treated suspension/counts per minute of control suspension.
[d] E < C, Suspensions treated with estradiol that had counts per minute less than those of control.

TABLE 6. *Effect of estradiol on uptake of tritiated thymidine by thymus and spleen*[a]

Concn (per ml)	Tissue	No. of suspensions tested	Counts per minute				% Change of mean
			Control suspensions		Estradiol-treated suspensions		
			Range	Geometric mean	Range	Geometric mean	
500 pg	Spleen	59	493–101,500	14,200	944–119,000	16,100	+13
500 pg	Thymus	15	4,250–137,000	32,800	7,600–119,000	38,400	+17
20 ng	Spleen	64	27,500–973,000	235,000	38,100–661,000	218,000	−7.2
20 ng	Thymus	67	72,600–6,000,000	552,000	68,700–1,670,000	492,000	−11

[a] 10^6 cells incubated for 24 h with 1 μCi of tritiated thymidine.

that concentration of estrogen is important in the effects which it has on lymphoid tissues. Small concentrations, in a range between 50 to 5,000 pg/ml, stimulate division of immunocompetent cells; slightly larger amounts suppress. This phenomenon may help explain the variation in results obtained by different investigators studying the effects of estrogen on antibody production, since various doses and various types of estrogen have been used. The concentration of estradiol may be particularly important in the effects it has on thymic-derived lymphoid tissue. Although small amounts of estrogen stimulate proliferation of thymic-derived cells, amounts no larger than 20 ng/ml seem to slightly suppress deoxyribonucleic acid synthesis and very large amounts have been shown to be thymolytic (20, 25, 26). Whitfield and his colleagues have shown a similar stimulation of mitosis of thymic cells by small amounts of cortisol, an effect which appeared to be due to sensitization of the cells to the action of cyclic adenosine 3′,5′-monophosphate (36). The molarities of the cortisol that produced the effect (10^{-8} and 10^{-9} M) are the same as those of estradiol, which stimulated mitosis in our studies. It appears that both cortisol and estradiol may stimulate or suppress proliferation of thymic-derived cells at low and high concentrations, respectively. However, physiological levels of estradiol in body fluids of females in the range of 10^1 to 10^3 pg/ml may be of a magnitude which generally enhance mitosis, whereas physiological concentrations of cortisol, 50 to 10,000 times greater, may tend to suppress cell division. The possibility that sex hormones, like corticoids, may affect concentrations of cyclic nucleotides within B and T cells or sensitize lymphoid cells to the action of cyclic nucleotide, and thus alter rates of mitosis and immunological responsiveness, must be further investigated.

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