Estrogen Pharmacology. II. Suppression of Experimental Immune Polyarthritis. (29715)

MARK N. MUELLER* AND ATTALLAH KAPPAS (Introduced by Leon O. Jacobson)
(With technical assistance of Evelyn Damgaard and Elizabeth Laves)
Department of Medicine, University of Chicago, and Argonne Cancer Research Hospital,† Chicago, Ill.

A generalized inflammatory joint disease develops in rats after intradermal injection of complete adjuvant. The studies of Pearson, Waksman and associates(1,2) suggest that this disease is immunological in nature having features of a delayed hypersensitivity type of reaction. The recent demonstration(3) that passive transfer of the arthritis can be effected by living lymphoid cells from sensitized donors strongly supports this view.

Natural estrogens significantly alter the morphology and function of hemopoietic and lymphoid tissues and may influence the occurrence and intensity of certain immunological reactions(4,5,6). In the present study it is shown that these steroid hormones significantly diminish the incidence of adjuvant-induced arthritis in rats.

Procedures and results. Sprague-Dawley rats 12 weeks of age or older were used throughout. Weights of males were approximately 300 to 400 g at time of inoculation; weights of females were approximately 200 to 300 g. Equal numbers of both sexes were used in each experimental group. Adjuvant was prepared by dispersing heat-killed Mycobacterium Butyricum (DIFCO) in light mineral oil (Bayol 55, ENCO) and administered as a single intradermal injection of 0.1 ml at the base of the tail. Steroids were dissolved in 0.5% N,N dimethylacetamide (Eastman Organic Chemicals) in propylene glycol. Steroid and appropriate solvent vehicle control solutions were sterilized by cold filtration and injected subcutaneously in the axillary fold in the amounts indicated in the Table. Animals were examined for arthritis on alternate days after adjuvant inoculation for 30 to 60 days. A separate control group treated with appropriate amounts of solvent vehicle alone was used in each experiment. The χ^2-test was utilized to evaluate the significance of the data(7).

Table I summarizes all experiments, indicating mode of treatment, number of animals observed, number and per cent of animals developing arthritis with an evaluation of the significance (p-values) of the difference between incidence of arthritis in the steroid treated groups as compared with the control group in each experiment.

Discussion. These experiments demonstrate that the natural hormone estradiol consistently diminishes the incidence of inflammatory joint disease evoked by adjuvant injection in rats. This protective effect of estradiol was apparent over a wide dose range and in a variety of treatment schedules (Exp. I-IV); moreover, estimation of the severity of the arthritis on the basis of number of inflamed joints and duration of involvement indicated that estradiol treated animals had much milder disease, as compared with controls. Estrone and estriol, *in vivo* transformation products of estradiol, were as effective in suppressing arthritis as the parent hormone. Since the transformation, estradiol → estriol, is not reversible *in vivo*, the protective effect of the latter steroid suggests, as in the hepatic action of these hormones(8), that other natural or synthetic C-18 steroids may have this property as well. Though not studied in detail, it appears likely on the basis of these (Exp. VI) and earlier observations(4), that the extent of estrogen protection depends in part on the intensity (*i.e.*, amount of Mycobacterium incorporated in the inoculum) of the antigenic challenge. The minimum amount of estrogen required for protection is not known with certainty, although in view of the ameliorative effect of gestation on this immunologic disease(9), it

* Present address: Dept. of Microbiology, Yale Univ. School of Med., New Haven, Conn.

† Operated by Univ. of Chicago for U. S. Atomic Energy Commission.

TABLE I. Treatment Schedules and Incidence of Adjuvant Arthritis in Rats Receiving Various Steroids.

Exp		M. Butyricum in adjuvant dose/animal (mg)	Group	Treatment	No. treated	No. with arthritis	% Arthritis	p value
I	A	.4	Control	—	47	33	70.2	
	B	.4	Estradiol	.5 mg TIW 3 wk pre- + post-inoculation	49	17	34.7	<.005
II	A	.6	Control	—	40	39	97.5	
	B	.6	Estradiol	1.0 mg q.d. 10 days pre- + TIW 4 wk post-inoculation	40	19	47.5	
	C	.6	"	1.0 mg q.d. 10 days pre- + TIW 1 wk post-inoculation	24	9	37.5	<.005
	D	.6	"	1.0 mg q.d. 10 days up to day of inoculation	32	15	46.9	
III	A	1.0	Control	—	20	12	60.0	
	B	1.0	Estradiol	.6 mg TIW 2 wk bgn. 5 days post-inoculation	13	3	32.1	<.025
	C	1.0	"	.6 mg TIW 2 wk bgn. 10 days post-inoculation	12	2	16.7	
IV	A	.6	Control	—	20	16	80.0	
	B	.6	Estradiol	.1 mg TIW 2 wk pre-inoculation	20	8	40.0	
	C	.6	"	.3 mg *idem*	22	7	31.8	<.01
	D	.6	"	.6 mg "	21	8	38.1	
	E	.6	"	1.0 mg "	22	7	31.8	
V	A	.6	Control	—	22	18	81.8	
	B	.6	Estradiol	1.0 mg TIW 3 wk pre-inoculation	22	10	45.4	
	C	.6	Estrone	*Idem*	22	6	27.3	<.005
	D	.6	Estriol	"	22	7	31.8	(estrogens
	E	.6	Cortisol	"	22	15	68.2	only)
	F	.6	Progesterone	"	43	31	71.8	
	G	.6	Testosterone	4.0 mg TIW 3 wk pre-inoculation	22	13	59.1	
	H	.6	Estradiol	1.0 mg TIW 4 wk from day of inoculation	22	8	36.4	
VI	A	.2	Control	—	30	9	30.0	
	B	.2	Estrone	1.0 mg q.d. 10 days pre-inoculation	30	—	.0	.005

The following abbreviations are used: TIW for 3 × per week; q.d. for daily.

is probably well within the range produced during normal rat pregnancy.

The incidence of arthritis evoked in rats treated with progesterone, testosterone and cortisol in the schedule and amounts shown in Exp. V was not significantly different from that in the control group. It should be noted that in this experiment, these hormones were administered prior to inoculation only, in order to compare their effects with those of the natural estrogens. According to the studies of Pearson and Wood(1), cortisol does diminish the severity of adjuvant arthritis when administered after inoculation.

The mechanism by which estradiol, estrone and estriol confer protection in adjuvant arthritis is not known. Since lymphocytes appear to be involved in the pathogenesis of this disease(2,3), the well-known depleting effects of estrogens on lymphoid tissues(10,11) may account in part for the protective action of these hormones. Another possible mechanism for this estrogen action is suggested by these experiments. It has been shown (12) that excision of the adjuvant injection site and its draining lymph nodes

shortly after inoculation does not prevent subsequent development of arthritis, indicating that the immunologic reaction leading to arthritis is quickly initiated. The fact (Exp. III and V) that estradiol diminishes the incidence of arthritis when administered in the latent period either starting on the day of inoculation, or 5 and 10 days thereafter suggests a hormonal effect directly on the capacity of involved tissues to react to the inflammatory stimulus in this disorder. Such an action would be consistent with earlier observations of Lurie(13), showing that both pregnancy and estrogens transiently suppress the intensity of delayed skin reactions in known tuberculin positive animals.

Estrogen administration and/or pregnancy has in other studies been shown to lessen the incidence and severity of experimental allergic thyroiditis, diminish the intensity and frequency of delayed skin reactions to thyroglobulin and tubercle protein and prolong the survival and function of trophoblast implants and of skin grafts in experimental animals and man(4,13,14,15,16). The present study with an inflammatory joint disease considered to be immunological in nature represents another experimental context in which estrogens are shown to suppress the manifestations of an apparent delayed type immune reaction, and which suggests that such hormones may mediate in part the related effects of pregnancy on such phenomena. The possibility that this estrogen action has clinical relevance may be considered in view of the extraordinary increase in estrogen production (principally estriol) which characterizes human pregnancy(17) and the profound ameliorative effect of gestation on both human(18) and experimental(9) polyarthritis.

Summary. Estradiol and its transformation products, estrone and estriol, markedly diminish the incidence and severity of the inflammatory polyarthritis induced by adjuvant injection in rats. These natural estrogens confer protection whether administered before inoculation or during the latent period of the disease, suggesting that they may act in part to suppress the capacity of the involved tissues to react to the immunologic inflammatory stimulus in this disorder. These observations are consistent with others which indicate that estrogens and/or pregnancy may alter certain expressions of immunological responsiveness in man and experimental animals.

1. Pearson, C. M., Wood, F. D., *Arthritis and Rheum.,* 1959, v2, 440.

2. Waksman, B. H., Pearson, C. M., Sharp, J. T., *J. Immunol.,* 1960, v85, 403.

3. Waksman, B. H., Pearson, C. M., Sharp, J. T., Wennersten, C., *Int. Arch. Allergy,* 1963, v23, 129.

4. Kappas, A., Jones, H. E. H., Roitt, I. M., *Nature,* 1963, v198, 902.

5. Kappas, A., Palmer, R. H., *Pharmacol. Rev.,* 1963, v15, 123.

6. Thompson, J. S., Reilly, R. W., Russe, H. P., Kappas, A., *Nature,* 1964, v203, 265.

7. Brownlee, K. A., *Statistical Theory and Methodology in Science and Engineering,* John Wiley & Sons, Inc., New York, 1961.

8. Mueller, M. N., Kappas, A., *J. Clin. Invest.,* 1964, v43, 1905.

9. Houssay, R. H., *Rev. Soc. argent. Biol.,* 1960, v36, 282.

10. Blecher, M., White, A., *Rec. Prog. in Hormone Res.,* 1959, v15, 391.

11. Dougherty, R. F., *Physiol. Rev.,* 1952, v32, 379.

12. Ward, J. R., Jones, R. S., *Arthritis and Rheum.,* 1962, v5, 557.

13. Lurie, M. B., *Ann. N. Y. Acad. Sci.,* 1950, v52, 1074.

14. Lajos, L., Görcs, J., *Nature,* 1962, v196, 178.

15. Andresen, R. H., Monroe, C. W., *Am. J. Obstet. & Gyn.,* 1962, v84, 1096.

16. Heslop, R. W., Krohn, P. L., Sparrow, E. M., *J. Endocrinol.,* 1954, v10, 325.

17. Fishman, J., Brown, J. B., Hellman, L., Zumoff, B., Gallagher, T. F., *J. Biol. Chem.,* 1962, v237, 1489.

18. Hench, P. S., *Proc. Staff Meetings of Mayo Clinic,* 1938, v13, 161.

Received September 2, 1964. P.S.E.B.M., 1964, v117.