

myocardial depression, the time element must play a dominant role.

Additional information regarding the nature of left ventricular depression induced by large doses of posterior pituitary extracts was obtained by an analysis of the aortic pressure pulses. Experimental evidence indicated that

serious cardiac consequences are not apt to result from use of posterior pituitary preparations administered to patients with normal hearts in therapeutic doses.

We desire to express our appreciation to Professor C. J. Wiggers for his supervision of the experiments and help in interpreting the data.

16000 P

Effect of Estrogens on Early Development of Frog Embryos.*

ROLAND K. MEYER, L. S. KOSMO, AND W. H. MCSHAN.

From the Department of Zoology, University of Wisconsin, Madison, Wisconsin.

Boell¹ showed that cytochrome oxidase activity undergoes changes during the development of *Amblystoma punctatum* embryos, and McShan and Meyer² reported that certain estrogens inhibit the succinoxidase system in mammalian tissues. This inhibition by the estrogens was shown to be mediated through the cytochrome oxidase of the system. In the light of these results and the fact that cytochrome oxidase plays an important role in respiration, it became of interest to determine whether exposure of fertilized frog eggs to natural and synthetic estrogens would affect their normal development. Eggs of *Rana pipiens* and *Rana clamitans* were treated with diethylstilbestrol and with estrone in varying concentrations and for varying periods of time. The results of these preliminary experiments will constitute the data presented in this paper.

Frogs were procured from a dealer and stored in the laboratory at 4°C until used. Ovulation was produced and the eggs fertilized according to the standard technique of Rugh.³

A 10⁻³ M stock solution of diethylstilbestrol was prepared as reported by McShan and Meyer, and a solution of estrone of the same strength was made by dissolving 2.7 mg of estrone in 0.3 cc of 2 N NaOH plus 0.2 cc of 95% alcohol and diluting to a volume of 10 cc. The required strength of the estrogens was obtained by dilution with 20% Holtfreter's solution.⁴ Proper control solutions of NaOH and alcohol were used.

The eggs were treated 1 hour after fertilization (after rotation and before the first cleavage) by dividing them into groups of 30 and placing them in 50 cc of the test solution. After exposure the eggs were rinsed with distilled water, transferred to Holtfreter's solution and maintained at 18°C for 5 days. Stages of development attained by normal and abnormal embryos were designated according to Shumway.⁵ Abnormalities were recorded by number from Rugh's series of photographs.³

In the first series of experiments eggs of *Rana pipiens* and *Rana clamitans* were exposed to diethylstilbestrol in concentrations of 27, 13.5 and 6.8γ per cc for periods increasing by half hours from ½ hour to 4 hours. Control groups of eggs were placed in 6/100,000, 6/200,000 and 6/400,000 M NaOH and in 20% Holtfreter's solution. In

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¹ Boell, Edgar J., *J. Exp. Zool.*, 1945, **100**, 331.

² McShan, W. H., and Meyer, Roland K., *Arch. Biochem.*, 1946, **9**, 165.

³ Rugh, Roberts, *Experimental Embryology*, 1941, N. Y. Univ. Bookstore, 18 Washington Place, New York.

⁴ Holtfreter, J., *Arch. f. Ent. Mech.*, 1931, **124**, 404.

⁵ Shumway, Waldo, *Anat. Rec.*, 1940, **78**, 139.

TABLE I.
Effect of Diethylstilbestrol and Estrone on the
Development of Eggs of *Rana pipiens*.

Treatment*	γ /cc	No. eggs	% abnormal	% survival
D	27.0	154	95.5	12.3
E	27.0	146	100.0	7.5
D	13.5	152	54.6	71.1
E	13.5	152	99.3	6.6
D	6.8	152	34.9	97.4
E	6.8	150	100.0	0
D	2.7	61	4.9	100.0
E	2.7	117	100.0	0.9
E	1.35	123	90.2	26.0
E	0.68	121	26.4	90.1

* Eggs were exposed to the solutions for a period of $2\frac{1}{2}$ hrs beginning 1 hr after fertilization. Development was observed for 5 days.

D = diethylstilbestrol. E = estrone.

R. pipiens, with only one hour's exposure, 27γ of diethylstilbestrol per cc produced approximately 100% abnormal embryos and high mortality; 4 hours with 6.8γ per cc produced less than 40% abnormality and low mortality. 13.5γ per cc for $2\frac{1}{2}$ hours, however, gave approximately 55% abnormality and 30% mortality. Eggs of *R. clamitans* showed a similar variation in response according to concentration and period of exposure but were more sensitive. 1 to 12% of control embryos were abnormal.

On the basis of these results the $2\frac{1}{2}$ hour period of exposure was used for a comparison of the effects of estrone and di-

ethylstilbestrol on eggs of *R. pipiens*. Concentrations ranging from 27 to 0.68γ per cc together with appropriate control solutions were employed. The data in Table I show that the effects obtained with estrone were much greater than those produced by diethylstilbestrol, and that when the ratio of abnormal embryos was about 50% or less the percentage survival was high. Abnormalities in the controls ranged from 1.3 to 10%. The stronger concentrations of both diethylstilbestrol and estrone produced a large number of abnormal embryos which did not develop beyond the blastula stage. Severe exogastrulation and various degrees of spinal bifida were the most common abnormalities observed.

Since it has been shown that estrogens inhibit cytochrome oxidase *in vitro*, further work is being done to determine whether this enzyme system can be inhibited in frog embryos by estrogens and thereby offer a possible explanation for the effects reported herein.

Summary. Abnormalities were produced in the developing eggs of *Rana pipiens* and *Rana clamitans* by exposure to diethylstilbestrol and to estrone in varying concentrations and for varying lengths of time. Higher concentrations of both substances produced more abnormalities, and estrone was found to be more effective than diethylstilbestrol.

16001 P

A Simple Method of Producing Control Guinea Pig Immune Sera for Use with Complement Fixing Antigens for the Arthropod-Borne Virus Encephalitides.*

W. MCD. HAMMON AND CARLOS ESPANA.†

From the George Williams Hooper Foundation for Medical Research, University of California, San Francisco, California.

It was noted in this laboratory that the sera of guinea pigs, following the mild in-

fection resulting from an intracerebral inoculation of St. Louis virus,¹ had good neutral-

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