could also explain the present results with sudden loss of mucosal serotonin causing intraintestinal loss of water. Cause of death in adrenalectomized animals receiving reserpine therefore is not as simple as that of normal rats receiving dicumarol and reserpine, or adrenalectomized rats receiving dicumarol, where there is a clear interference with 2 mechanisms of hemostasis simultaneously. However, it seems reasonable to accept that part of the situations which are common—increased capillary fragility and serotonin displacement, contribute to the hemorrhage. The marked gastrointestinal activity of reserpine in the adrenalectomized animal will mean this must be main site of hemorrhage, and the fact that platelet physiology has been disturbed may possibly mean that when intraintestinal hemorrhage occurs, the platelet plugging required for hemostasis cannot follow.

Summary. Reserpine given to adrenalectomized rats produced diarrhea and gastrointestinal hemorrhage with high mortality. This was not observed in sham-operated animals. Maintenance on desoxycorticosterone protected the animals from this action of reserpine.

1. Gaunt, R., Renzi, A. A., Antonchak, N., Miller, G. J., Gilman, M., *Ann. N. Y. Acad. Sci.,* 1957, v59, 27.

2. Pletscher, A., Shore, P. A., Brodie, B. B., *J. Pharm. & Exp. Ther.,* 1956, v116, 84.

3. Jaques, L. B., Mogenson, G. J., Fisher, L. M., *Trans. Roy. Soc. Can.,* 1956, v50, 9.

4. Jaques, L. B., Fisher, L. M., *Arch. Int. de Pharmacodyn.,* 1959, v120, 351.

5. van Cauwenberge, H., Jaques, L. B., *Thrombosis et Diathesis Haem.,* 1959, v3, 45.

6. Correll, J. T., Lyth, L. F., Long, S., Vanderpoll, J. C., *Am. J. Physiol.,* 1952 v169, 537.

7. Zucker, M. B., Borrelli, J., *Am. J. Clin. Path.,* 1956, v26, 13.

8. Plummer, A. J., Earl, A., Schneider, J. A., Trapold, J., Barrett, W., *Ann. N. Y. Acad. Sci.,* 1954, v59, 13.

9. Kramar, J., Meyers, V. W., Kramar, M. Simay, Wilhelmj, C. M., *Am. J. Physiol.,* 1956, v184, 640.

10. Selye, H., Heuser, G., 5th Ann. Rept. on Stress, 1955-1956, p153.

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Cytostatic Activities of Steroidal Estrogens against Zebra-Fish Embryos. (25775)

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To find a superior estrogen for chemotherapy, we have studied a large list of steroidal estrogens for their cytostatic effects on the egg of the zebra-fish (*Brachydanio rerio, Hamilton*). Of the 14 steroidal estrogens studied, we have also tested the 3-methyl and 3-ethyl ethers of each. Some of these ethers are reported here for the first time.

Materials and methods. The method was a modification of one previously described(1). Our present practice is to use 10 embryos (8-64 cell stages) in 50 ml of culture solution exposed at 26°C for 24 hrs. Each test culture is replicated and at least 3 replicated tests are used to determine minimum concentration (in parts per million) of the steroid which affects 50% of the embryos (ED/50/24). Thus, a minimum of 50-60 embryos was used in testing each concentration.

Results. The results are summarized in Table I.

Discussion. As measured in the restraint of cell division in the freshly-fertilized zebra-fish egg, 17-ketosteroids are in general less cytostatic than 17-hydroxysteroids. The one exception is equilenin, which produced a curious type of cytostasis. The equine estrogens

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TABLE I. Comparative Cytostatic Activities of Steroidal Estrogens on the Zebra-Fish Embryo.

		ED/50/24		
	Estrogen	Free phenol, ppm.	3-Methyl ether, ppm.	3-Ethyl ether, ppm.
Bi-functional	Estrone-17 (U.S.P.)	Inactive	2–4	1–2
	Estradiol-17β (U.S.P.)	2–3	1–2	.5–1
	" -17α	2	2–3 (D)	.5–1 (D)
	Estrone-16	Inactive	2–4	Inactive
	Estradiol-16β	2	.5–1	"
	" -16α	1	1–2	"
	Equilin	Inactive	1–3 (D)	" (>20)
	17-Dihydroequilin-17β	1–2 (D)	1–2	.5
	Equilenin	.5–1 (D)	Inactive	Inactive
	17-Dihydroequilenin-17β	.5–1 (D)	.5–1 (D)	1–2 (D)
Tri-functional	16-Keto-estrone	Inactive	Inactive	2–3
	16-Keto-estradiol-17β	"	"	Inactive
	16-Epiestriol	"	"	"
	Estriol	"	"	"

ED/50/24 means concentration in which 50% of embryos show effects of drug after 24 hr of exposure. Unless indicated otherwise, effects are considered to be cytostatic. This is usually indicated by death of embryo or abnormal development. In cases indicated by (D) effect is retardation of development. *Inactive* means that ED/50/24 was clearly greater than 2 ppm. Often partial insolubility of steroid results at concentrations greater than 2 ppm.

often caused only retardation of development with no lethality.

Bi-functional estrogens are considerably more active than tri-functional estrogens. This is in curious contrast to the situation which obtains in measurement of the anti-mitotic activity against the chick-embryo fibroblast in tissue culture. We have reported(2) the extremely powerful effects of certain tri-functional estrogens. Estriol, however, had very weak anti-mitotic effects against the fibroblast.

16-Oxygenated estrogens were found to be more cytostatic than 17-oxygenated estrogens when the steroidal C_3 position is hydroxyl or methoxyl; this situation, however, is reversed in the case of 3-ethoxyl substitution.

In agreement with the findings of Druckrey, Danneberg and Schmähl(3), who used the sea urchin egg, we have found no relationship whatever between estrogenic hormone potency and cytostatic potency. As an illustration, the 3-methyl ether of estradiol-16β is 3 to 4 times more cytostatic than natural estradiol, but the latter is at least one thousand times the more potent by the Allen-Doisy assay.

Summary. The cytostatic effects of 14 steroidal estrogens and their 3-methyl and 3-ethyl ethers have been studied using the embryo of zebra-fish (*Brachydanio rerio*) as test object. Of estrogens tested, 3-ethyl ether of 17-dihydroequilin-17β was the most active.

1. Jones, R. W., Huffman, M. N., *Trans. Am. Micro. Soc.,* 1957, v76, 177.

2. Huffman, M. N., Jones, R. W., Katzberg, A. A., *Cancer,* 1957, v10, 707.

3. Druckrey, H., Danneberg, P., Schmähl, D., *Arzneimittel-Forsch.,* 1953, v3, 151.

Received March 3, 1960. P.S.E.B.M., 1960, v104.