formol-saline is added for each slant. In vaccinating guinea pigs, a total of 4 cc of vaccine is administered subcutaneously in doses of one, one, and 2 cc at 5-day intervals. Animals so treated are found to be solidly immune when tested a month later with blood virus (Chart 1). Serum from 2 vaccinated pigs taken 3 weeks after vaccination agglutinated spotted fever rickettsiae (chick tissue slants) in dilution of 1/20+++, 1/40++, 1/80+. These 2 sera also protected normal guinea pigs when 1 cc was mixed with passage blood and injected after standing 40-60 minutes at room temperature.

10854

Estrogenic Properties of Extracts of Ovaries of Certain Marine Invertebrates.*

J. KENNETH DONAHUE. (Introduced by W. W. Swingle.)

From the Department of Biology, The College of Charleston, and the Bermuda Biological Station for Research, Inc.

The writer has previously reported that extracts of the ovaries of a Bermuda echinoderm *(Lytechinus variegatus)* caused considerable growth of the epithelium of the uterus and vagina when injected into the mature, ovariectomized rat.[1] However, failure of the uterus to swell with fluid as it does in full normal estrus indicated that the response was subthreshold and, possibly, that the stimulating agent in the extracts differed in some respects from estrogens derived from mammalian sources. The present study was undertaken in order to test for estrogens in other invertebrate ovaries and to make further observations of the effects of the ovarian extracts upon the vaginal and uterine epithelium of the rat.

The ovaries of the common sea urchin *(Lytechinus variegatus)*, the reef urchin (Echinometria), the holothurian *(Stichopus mobii)*, and the lobster *(Palinurus argus)* were collected in Bermuda during the summer of 1938 in considerable quantities. In all cases the ovaries were sexually mature or were rapidly approaching that state when collected. After removal the ovaries were drained of as much sea water as possible and then thoroughly ground in a mortar. The ground tissues were then placed in separate containers of 95%

* The expenses of this investigation were defrayed in part by a grant from the Penrose Fund of the American Philosophical Society.

1 Donahue, J. K., and Jennings, E. D., *Endocrinology*, 1937, **21**, 5.

alcohol. Extraction work was carried out in Charleston the following autumn and winter. The ovarian material was extracted 3 times with 5 times its volume of 95% ethyl alcohol. Each of the extraction periods lasted for 48 hours during which time the mixture was shaken frequently. The filtrates were combined and concentrated by distillation under reduced pressure. The tissue residues were extracted in a similar manner with acetone and petroleum ether and concentrated. The heavy, oily concentrates were combined and used either in this form or mixed with various dilutions of corn oil. This procedure was carried out for all of the species employed. All extracts, with the exception of the one obtained from Palinurus, gave positive Salkowski reactions indicating the presence of sterols. In the dosages employed the extracts were nontoxic.

Mature, ovariectomized rats of the Wistar strain were used as test animals. Following ovariectomy they were left untreated for 10 days. With the previous knowledge that such extracts contain, at best, but small quantities of estrogens several modes of administration were adopted in order to ascertain that which would give a maximal response. In some cases subcutaneous injections were given throughout the standard 10-day injection period (designated "s.c" in Table I). In others subcutaneous injections were given every other day and direct applications of extract into the vagina made on alternate days (designated "s.c.-v." in Table I). Finally, some of the animals received direct applications of extract into the vagina throughout the period of treatment (designated "v." in Table I). All animals were killed on the 11th day and the uteri and vaginæ

TABLE I.
Effect of Invertebrate Estrogens on the Vagina and Uterus of the Rat.

Rat and source of estrogen	Age in days	Daily dosage: extract, cc	Daily dosage: corn oil, cc	Route	Effect on vagina*	Effect on uterus	Injection period, days
S 1	190	.25	.25	s.c.	none	none	10
S 2	190	.4	.1	s.c.-v.	12	hyperemic	10
S 3	80	.25	.25	v.	8	"	10
S 4	80	.4	.1	v.	9	"	10
E 1	190	.25	.25	s.c.	3	slight	10
E 2	190	.4	.1	s.c.-v.	12	hyperemic	10
E 3	90	.25	.25	v.	5	"	10
P 1	190	.25	.25	s.c.-v.	4	slight	10
P 2	90	.25	.25	v.	5	"	10
L 1†	190	.25	.25	v.	0	none	10

"S"—Stichopus, "E"—Echinometria, "P"—Palinurus, "L"—Lytechinus.
* Figures indicate approximate number of newly grown cell layers in vaginal epithelium.
† This extract 1½ years old.

fixed for histological study. Vaginal smears were taken once daily during the entire experimental period, great care being exercised not to traumatize the vagina. When the extract was applied directly into the vagina the animal was held head downward and the extract gently forced into the vagina with a pipette. The results of this procedure are summarized in Table I.

The smear records followed fairly identical courses in those cases in which positive reactions occurred. After the third day of treatment a marked diminution of leucocytes and the appearance of large numbers of nucleated epithelial cells and cornified epithelial cells was noted. In 3 cases full estrous smears appeared on the 7th or 8th day (E2, P1, and S2). In the majority of cases the reappearance of leucocytes around the 10th day was noted, indicating that the establishment of a growth phase in the vaginal epithelium was not as permanent as is the case when standard estrogens are given. As the table indicates, the uteri of the positively reacting animals showed some hyperemia but no gross signs of swelling with fluid even in those instances where full estrous smears were obtained. For controls 3 ovariectomized rats were injected with 0.5 cc of corn oil daily for 10 days; 3 received alternate injections and vaginal applications of the same amount of corn oil daily for 10 days; and 3 received vaginal applications of corn oil daily for 10 days. In no case did this treatment disturb the diestrous phase resulting from ovariectomy. At this point quantitative data include only the yield of crude extract from equivalent amounts of ovarian tissue in the dry state. Ninety cc of Stichopus extract represented 90 g of dried ovarian tissue. Two hundred cc of Echinometria extract represented 55.5 g of tissue and 49 cc of Palinurus extract represented 25.5 g of tissue.

It is concluded that all of the extracts tested contained estrogenic material. Unfortunately injections of this type of extract amounting to more than 0.5 cc per day are not well tolerated. It is likely that higher dosages would have given full estrous reactions in a larger number of animals. It appears from these results that estrogenic materials from this source have a specific effect upon cell growth of the vaginal epithelium and, to a lesser extent, upon the epithelium of the uterus. However, they do not effect the fluid shift which usually occurs into the lumen of the uterus in estrous produced in other ways. The suggestion is made that estrogenic substances from this source may not contain the chemical complex responsible for the uterine edema found in normal estrous rats.