

repeat this experiment we² found that effective amounts of estrone may be transmitted from animal to animal. The animals used in this experiment were kept segregated as a group throughout the experiment. They were spayed. No accessory ovaries were found at autopsy. Smears were made twice daily for 5 days before treatment was begun; and they were all negative. Smears were omitted until 11 weeks after treatment began, to avoid pseudo-estrus³ effects from mechanical stimulation of the vagina. Smears were made twice a week throughout the rest of the experiment, which terminated 41 weeks after treatment with 1:2:5:6 dibenzanthracene was begun. If a positive or plus-minus smear was found all animals were smeared daily until there was a return to negative. Two untreated spayed controls were kept in the box with the treated mice. Later 4 old spayed females with uterine implants of dibenzanthracene were segregated and kept as additional controls. They were caged separately. Table I shows the positive vaginal smears. Periods when all were negative or plus-minus are omitted for brevity. Estrus was occasionally observed in the treated animals and in the controls.

Frank⁴ has reported positive vaginal smears in human post-menopausal and castrate cases. Loeb⁵ has reported hyperplasia of the epithelium of the cervix and vagina of old mice. These data suggest that occasional definitely positive vaginal smears develop in old or sexually involuted females. Whether the response is to local or general stimuli is unknown, as is its significance.

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Estrogenic Activity of 3-4 Benzpyrene.*

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Cook and Dodds¹ reported that 3 of the carcinogenic hydrocarbons were estrogenic. The carcinogenic and estrogenic effects were

² Perry, I. H., *Proc. Soc. Exp. Biol. and Med.*, 1937, **36**, 122.

³ Emery, F., and Schwabe, E. L., *Anat. Rec.*, 1936, **64**, 147.

⁴ Salmon, U. J., and Frank, T., *Proc. Soc. Exp. Biol. and Med.*, 1936, **33**, 621.

⁵ Burns, E. L., Suntzeff, V., Muskup, M., and Loeb, L., *Am. J. Ca.*, 1938, **32**, 534.

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¹ Cook, J. W., and Dodds, E. C., *Nature*, 1933, **131**, 205.

not quantitatively or temporally related. A causal relationship is a matter for speculation. Of these 3 estrogenic carcinogens 3-4 benzpyrene has been the most extensively experimented with. Cook and Dodds injected 100 mg of the carcinogenic hydrocarbon in oil subcutaneously into each of 10 rats. Three of the 10 rats receiving 3-4 benzpyrene had positive vaginal smears for 150 hours.

Carminati² reported that 3-4 benzpyrene was not estrogenic. He used the same dose as Cook and Dodds for 2 rats, and various smaller doses for 9 other rats.

We injected 31 mg of 3-4 benzpyrene in 0.75 cc of lard subcutaneously into each of 4 mice standardized according to Palmer's³ method. In 48 hours 3 had positive vaginal smears. On the fifth day the fourth mouse had plus-minus reaction. On the sixth day all were negative, and remained so. Twenty spayed but not standardized mice were injected subcutaneously with 4 mg of 3-4 benzpyrene in lard. They did not develop estrus. Much of the injection mass was present in the 2 mice dying without tumor and a moderate amount present in those developing tumors so the estrogenic amount must lie between 31 and 4 mg.

One of the 4 animals receiving 31 mg of 3-4 benzpyrene died in the third week. A second animal died at 2½ months. Neither of these had tumors. One month after being injected 2 animals developed tumors at the site of injection. One of these animals was the one that did not go into estrus. The other was not in estrus when the tumor began. The animals were killed at 3½ and 4½ months when the tumors measured about 4x3x3 cm. The tumors were sarcomas with considerable cytological variation. There were no metastases. Five to 10 mg of benzpyrene subcutaneously produces tumors in about 3½ months.⁴ The development and duration of the tumors in the massively injected mice does not appear to vary significantly from the response to smaller doses.

Three controls injected with 1 cc of lard did not go into estrus or develop tumors.

² Carminati, V., *Tumori*, 1935, **9**, 106.

³ Palmer, Allan, *PROC. SOC. EXP. BIOL. AND MED.*, 1937, **36**, 123.

⁴ Fieser, L. F., Fieser, Mary, Hershberg, E. B., Newman, M. S., Seligman, A. M., and Shear, M. J., *Am. J. Cancer*, 1937, **29**, 260.