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Absorption of Crystalline Estrogen from Paraffin Nodules in the Rat.*

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Several methods have been devised to avoid the waste necessarily ensuing from the rapid absorption and excretion of the sex hormones when these substances are injected in oil. Deanesly and Parkes¹ inserted compressed tablets of the crystalline hormones subcutaneously, Noble² used a similar method for introducing synthetic estrogens, while Brock and Druckrey³ placed intraperitoneally collodion sacs containing crystalline estrone. Emmons⁴ studied the absorption from compressed tablets inserted subcutaneously, and found minimal variation in the rate for each hormone. With these technics small quantities of hormone were effective for considerably longer periods than is the case when similar amounts are administered in solution.

Geschickter,⁵ who reported the occurrence of mammary cancer in the rat approximately 300 days following the subcutaneous implantation of a pellet containing 7.5 mg estrone, stated that it required 4 to 7 months to absorb completely one weighing 3 mg. Mammary lesions resembling neoplasia, which appeared in the rat after a minimum of 226 days, have been described by Noble, McEuen, and Collip⁶ as a result of absorption from a compressed tablet of estrone weighing 1 to 7 mg, but the hyperplastic tumor-like lesions regressed following removal of the hormone, or treatment with progesterone.⁷

An effective method for the quantitative estimation of the potency of carcinogenic hydrocarbons has been employed in this Department for a number of years. The carcinogens are dissolved in

* Estradiol dipropionate was generously furnished by Dr. R. MacBrayer, Ciba Pharmaceutical Products, Inc., Summit, N.J.

¹ Deanesly, R., and Parkes, A. S., *Proc. Roy. Soc. B.*, 1937, **124**, 279.

² Noble, R. L., *The Lancet*, 1938, **2**, 192.

³ Brock, N., and Druckrey, H., *Klin. Wchnschr.*, 1938, **17**, 23.

⁴ Emmons, C. W., *Endocrinology*, 1941, **28**, 633.

⁵ Geschickter, C. F., *Radiology*, 1939, **33**, 439.

⁶ Noble, R. L., McEuen, C. S., and Collip, J. B., *Canad. M. A. J.*, 1940, **42**, 413.

⁷ Noble, R. L., and Collip, J. B., *Canad. M. A. J.*, 1941, **44**, 1.

paraffin wax (melting point 48 °C), which is injected subcutaneously in the fluid state. The material solidifies almost immediately as fairly uniform ellipsoids. Statistical analysis of the results support the concept that malignant disease is a local manifestation, a consequence of the direct action of a carcinogen producing a specific cellular injury.^{8, 9}

Hyperplastic mammary alterations were prominent about methylcholanthrene foci in rats and a few mammary carcinomas were produced locally.⁸ It was considered probable that only minimal absorption of the hydrocarbons took place, and induced tumors at a distance from the injected material were never observed. No attempt was made to compute the rate of absorption from the nodules, but to judge from the color of those which had lain in animals for long intervals it may be estimated that, regardless of the presence of a tumor, a considerable quantity of the carcinogen was retained locally. That some absorption occurred, however, was evident from the fact that increasing doses of methylcholanthrene caused increasingly high mortality.

Experimental. The present communication deals with the absorption and utilization in rats of crystalline estradiol dipropionate incorporated in paraffin. The method has the advantage of simplicity, rapidity, and effectiveness. The estrogen dissolves readily in the proportion of 5 g per 100 cc of paraffin, which is kept liquid in a water bath at 70°C. As 0.1 cc of this solution is administered, the animals receive 5.0 mg crystalline estrogen. Injections are carried out with a tuberculin syringe having a short, large bore needle, likewise kept warm in a test tube immersed in a water bath.

The material was injected subcutaneously in the flanks, thoracic region, or back, intramuscularly in the thigh, or intraperitoneally. Minimal reaction developed about the solidified masses of paraffin, and the ellipsoids eventually became encysted in a thin layer of relatively acellular connective tissue. Some organization occurred in the interior of the nodules. More intense changes suggesting local malignant transformation in the surrounding fibrous tissue have not been observed. In the peritoneal cavity the nodules were generally situated in the greater omentum and here also produced minimal local effects. The location of the nodules exerted no influence on the activity of the hormone, as determined by general influences or on the rate of its utilization.

⁸ Dunning, W. F., Curtis, M. R., and Eisen, M. J., *Am. J. Cancer*, 1940, **40**, 85.

⁹ Dunning, W. F., Curtis, M. R., and Wood, F. C., *Am. J. Cancer*, 1940, **39**, 70.

While the results of some investigations^{10, 11, 12} appear to indicate that estrogen is inactivated by the liver, the present observations, those of Brock and Druckrey,³ and of Biskind and Mark,¹³ who found that estrone pellets placed in the gastric omentum or the liver were effective, demonstrate that inactivation does not necessarily take place when the total implanted hormone first passes through the liver.

Absorption from the paraffin nodules occurred rapidly, as shown by the production of complete estrus in 48 to 72 hours in infantile rats. Animals 3 to 5 weeks of age of the inbred Sherman strain were employed. With constant absorption of the estrogen during succeeding periods the general effects became readily apparent. The animals were undersized, diffuse mammary hyperplasia was prominent, and ovarian function remained in abeyance, but this was frequently associated with enlargement and squamous cell metaplasia of the uterus, and pyometra. Hyperplasia of the anterior lobe of the pituitary developed relatively late, and was not of the magnitude induced by quickly absorbed oily solutions of the female sex hormone.

Vaginal smears were made weekly. An abrupt disappearance of the estrus smear was assumed to coincide with the complete absorption and utilization of the estrogen. As the object of this experiment was primarily the study of the lesions produced by slowly absorbed estrogen, no additional assays for hormone were conducted on nodules which were no longer effective in animals. In the absence of irreversible damage, as pyometra, other alterations regressed in rats which had utilized completely the estrogen, and normal ovarian function eventually supervened if age did not bar this possibility. Insertion of a new supply of estrogen in paraffin in animals which had reverted to a resting stage again produced the characteristic smear of full estrus. Thus, absorption of a physiologic substance, estrogenic hormone, from a paraffin nodule appeared to proceed more readily than absorption from a nodule containing a carcinogenic hydrocarbon, probably less easily soluble in the body fluids.

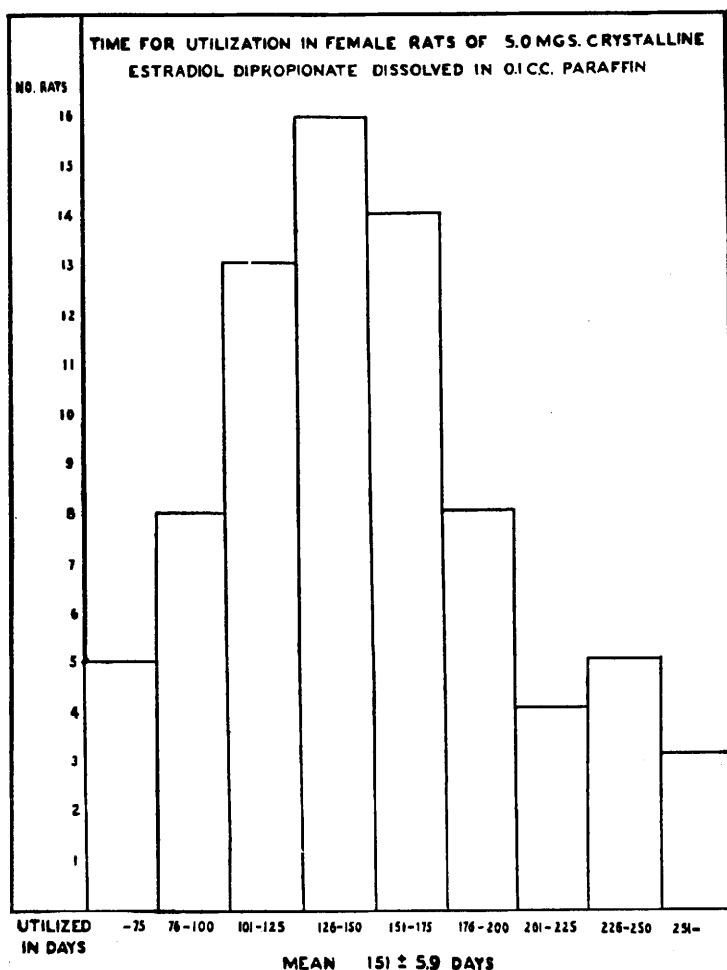
The range of variability in the utilization of the 5.0 mg of crystalline hormone is illustrated in the accompanying chart. The earliest negative smear occurred after 48 days, the latest after 301, the mean time for complete utilization being 151 ± 5.9 days.

¹⁰ Biskind, G. R., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **47**, 266.

¹¹ Golden, J. B., and Severinghaus, E. L., *PROC. SOC. EXP. BIOL. AND MED.*, 1938, **39**, 361.

¹² Zondek, B., and Sklow, J., *PROC. SOC. EXP. BIOL. AND MED.*, 1941, **46**, 276.

¹³ Biskind, G. R., and Mark, J., *Bull. Johns Hopkins Hosp.*, 1939, **65**, 212.



In addition to the 76 rats which utilized the estrogen completely, 8 died before the appearance of the first negative smear, while 38 succumbed between 57 and 318 days without utilizing the implanted hormone completely, as was evident from a persisting positive smear.

In 112 males employed, the size and position of the testes and histologic evidence of activity in the seminiferous tubules afforded a comparable indicator of the absorption and utilization of the female sex hormone. The animals used were littermates of the females and received simultaneously similar treatment. The undescended testicles remained rudimentary in size and function. However, after intervals comparable with those observed in females, complete utilization of the hormone became apparent from increasing

development of the testes. They now appeared externally, increased gradually to normal size, and eventually descended. Microscopic examination at this time disclosed normally progressing spermatogenesis and normal epithelium in the seminal vesicles. Nevertheless, if males that had utilized completely their first dose of estrogen were given an additional supply in paraffin, the testes gradually decreased in size and reverted to an intraabdominal position and a rudimentary functional state. Some observations on the temporary character of the testicular atrophy following treatment of males with estrogen have been reported by Deanesly,¹⁴ and on reversible changes in females by Grumbrecht.¹⁵

Summary. An effective and simple method of insuring slow continuous absorption of estrogen in the rat is the incorporation of the crystalline hormone in paraffin (melting point 48°C), which after subcutaneous, intramuscular, or intraperitoneal injection in a liquid state, solidifies locally. As indicated by vaginal smear, the average time required for complete absorption and utilization of 5.0 mg estradiol dipropionate contained in 0.1 cc paraffin, introduced into animals 3 to 5 weeks of age, is 151 ± 5.9 days. In males the position and activity of the testes are comparable indicators of the utilization of the injected estrogenic hormone.

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An Enzyme Which Inactivates Estrone.*

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Zondek¹ was able to demonstrate that liver pulp of homothermal and poikilothermal animals as well as mash of hyacinth roots² inactivate estrogenic hormone *in vitro*. Cell-free liver extract yielded similar results. The inactivating factor in liver and hyacinth roots

¹⁴ Deanesly, R., *J. Endocrinology*, 1939, **1**, 36.

¹⁵ Grumbrecht, P., *Arch. f. Gynäk.*, 1940, **170**, 1.

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¹ Zondek, B., *Lancet*, 1934, **227**, 356; *Skandin. Arch. Physiol.*, 1934, **70**, 133; *Hormone des Ovariums und des Hypophysenvorderlappens*, 2nd ed., Springer, Vienna, 1935, 124-162; *Genital Functions and Their Hormonal Regulations*, Williams & Wilkins, Baltimore, 1941, 147-175.

² v. Euler, H., and Zondek, B., *Biochem. Z.*, 1934, **271**, 64.