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Transplantation of Pituitary "Mammotropic" Tumor ($MtT.F_4$) from Fischer to Sprague-Dawley Rats.*† (31058)

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The $MtT.F_4$ pituitary tumor was originally induced by diethylstilbestrol treatment in inbred Fischer rats(1,2). This tumor was initially dependent on estrogen, since it could be transplanted only in estrogen-treated rats. After 4-5 serial passages in the Fischer rat, it became autonomous and could be transplanted and grow without estrogen treatment (2). The $MtT.F_4$ has been shown to secrete large amounts of prolactin, growth hormone (GH) and adrenocorticotropin (ACTH), but apparently no FSH, LH or TSH(3,4).

Earlier attempts to transplant $MtT.F_4$ in other strains of rats have been unsuccessful (Furth, personal communication). Since the $MtT.F_4$§ available to us had already undergone 40-41 passages, about half of them in our laboratory, it was of interest to determine whether by this time it could be transplanted successfully into randomly bred Sprague-Dawley (S-D) rats. The S-D strain was selected because it is not related to the Fischer strain (Dr. W. Dunning, personal communication), and because mammary tumors can easily be induced in this strain of rats by chemical carcinogens(5). Also, the Sprague-Dawley strain is much more readily available and less expensive to purchase than the Fischer strain.

Methods. S-D female rats, 7-8 weeks old, were obtained from Hormone Assay Lab., Chicago, Ill. Inbred female Fischer rats (CDF strain), approximately 75 days old, were obtained from Charles River Breeding Labs., Brookline, Mass. $MtT.F_4$ (passages 40 and 41) were transplanted subcutaneously, in the back of the neck. The tumors were removed by sterile technique, cut into fine pieces with iris scissors, and injected in a 0.1-ml volume of medium 199 (pH 7.4). In one group of S-D rats, each was injected subcutaneously with 10 μg estradiol in corn oil, 3 times weekly, beginning one day before tumor transplantation. In another group of S-D rats, each was injected subcutaneously with 50 IU PMS (Upjohn Co., Kalamazoo, Mich.), and 3 days later with 25 IU HCG (Nutritional Biochemicals Corp., Cleveland, Ohio); on the following day they were each given a tumor transplant.

Beginning 3 weeks after tumor transplantation, each rat was palpated once weekly for tumor development. The average time at which 50% of the rats from each group developed palpable tumors was considered to be the mean latency period. At termination of the experiment, approximately 10 months after tumor transplantation, the surviving rats were killed and all tumors were removed and examined histologically. The mammary glands, adrenals and other internal organs were also examined. Animals with palpable

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TABLE I. Transplantation of MtT.F_4 into Fischer and Sprague-Dawley Rats.

No. of rats	Treatment	No. of rats with tumors	% of rats with tumors	Mean latency (wk)
	Fischer rats			
12	Saline	12	100	4
	Sprague-Dawley rats			
12	Saline	5	50	7
12	Saline	7		8
10	Estradiol	6	50	8
12	PMS & HCG	5		7

tumors which died earlier were similarly examined.

Results. The results are summarized in Table I. Transplantation of the MtT.F_4 in the 12 Fischer rats was 100% successful, with a mean latency period of 4 weeks. This is in agreement with our previous reports(6, 7). By contrast, only 41-60% of the 46 S-D rats developed palpable tumors, and the mean latency period was 7-8 weeks. Neither estradiol nor PMS and HCG had any effect on tumor incidence in the S-D rats, suggesting that growth of the MtT.F_4 was independent of ovarian influences. The tumors in the S-D rats grew very slowly, and even after 5 months, never attained more than about ⅓ the size of the tumors in the Fischer rats. By this time, most of the S-D rats began dying of respiratory infections.

Histological examination of the tumors in the S-D rats revealed that they in no way differed from the original tumor transplants in the Fischer rats. The cells appeared to be monomorphous and chromophobic. Also, the hormones secreted by the MtT.F_4 in the S-D rats appeared to be similar to those secreted by the tumor in the Fischer rats. Greatly enlarged adrenals (4-6-fold increase in size) and almost complete disappearance of the thymus indicated ACTH release. The large increases observed in the size of the liver, kidney and spleen suggested GH effects, while intense mammary development and secretion indicated prolactin secretion. Most of the S-D rats with an MtT.F_4 had stomach ulcers, a phenomenon we have not observed previously in Fischer rats carrying MtT.F_4 tumors. These appeared to occur mainly in the fundic portion of the stomach.

Discussion. These results demonstrate that the MtT.F_4 pituitary tumor, originally induced in highly inbred Fischer rats, can be successfully transplanted to randomly bred S-D rats. Tumor incidence in the S-D rats was lower and the tumors did not reach the size of those transplanted into Fischer rats. Administration of estrogen or PMS and HCG did not increase the incidence or growth of the tumors in the S-D rats. Prolactin, GH and ACTH apparently were secreted by the MtT.F_4 in both strains of rats as indicated by intense mammary growth, marked increase in size of liver, spleen, and kidneys, and a 4-6-fold increase in adrenal size. The histological appearance of the tumor in both strains of rats was similar.

The ability of the MtT.F_4 to grow in the S-D rat may be related in part to its secretion of large amounts of ACTH, which stimulates release of high levels of adrenal cortical hormones. The latter could depress normal immunological barriers and permit growth of the MtT.F_4 tumor. This explanation does not appear to be entirely satisfactory, however, since previous attempts to transplant pituitary tumors from inbred strains of rats to other strains have not been successful. It is also doubtful whether sufficient ACTH would be released by the small amount of MtT.F_4 present at time of transplantation to alter adrenal cortical secretion. It is more probable that successful transplantation of the MtT.F_4 tumor to the S-D rat depends on its degree of autonomy and number of passages it had previously undergone. An MtT.F_4 which had undergone but few passages in the Fischer rat may not be transplantable to other strains. Whether the MtT.F_4 after many passages can be transplanted to other strains of rats has not yet been determined, but the MtT.F_4 is readily transplanted from S-D to S-D rats. The wide availability of S-D rats as compared to Fischer rats should facilitate the study of neuroendocrine influences on growth and hormone secretion by the MtT.F_4.

Summary. The MtT.F_4 "mammo-somatotropic" pituitary tumor, originally induced and maintained in highly inbred Fischer rats by Dr. J. Furth, was successfully transplanted into randomly bred S-D rats. The tumors

grew more slowly, the mean latency period was about twice as long (7-8 weeks as compared to 4 weeks), and about 41-60% of the S-D rats developed palpable tumors as compared to 100% in Fischer rats. Neither estradiol nor PMS and HCG altered tumor incidence in the S-D rats. The MtT.F_4 readily transplanted from S-D to S-D rats. As in Fischer rats, large amounts of prolactin, growth hormone and ACTH were secreted by the MtT.F_4 in S-D rats, as indicated by intense mammary stimulation; enlargement of the liver, kidneys and spleen; and a 4-6-fold increase in adrenal size. Unlike the Fischer rat, stomach ulcers were found in most Sprague-Dawley rats with successful tumor transplants.

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Sexual Receptivity and Fertility of Female Rats that are in Androgen Induced Persistent Vaginal Estrus. (31059)

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It has been amply demonstrated that neonatal androgen treatment of female rats will cause persistent vaginal estrus*(1). Limited evidence is also available to show that, depending upon the dose, some androgen-treated female rats will copulate but with unreliable frequency. Furthermore, these females which do mate continue to exhibit persistent vaginal cornification, and autopsy shows they have not ovulated(2). Daily cervical stimulation does not induce pseudopregnancy in androgen-sterilized rats(3), even though, as Everett(4) reported, persistent vaginal cornification is interrupted and ovulation takes place in spontaneous persistent estrous rats which copulate.

The work reported here was undertaken to establish if a small dose of androgen given to neonatal rats would produce sterile females with nymphomania.

Materials and methods. One hundred and fifty female Spartan (Sprague-Dawley strain) rats were injected s.c. with 10 μg of testosterone propionate (TP) in 0.05 ml of peanut oil on the 5th day after birth (day of birth called day 1). These females were weaned at 21 days, placed 5 to a cage and maintained at 72° ± 2°F with 14 hours of daily artificial light. Untreated virgin females 70 to 120 days old constituted the control group. Rats taken from these two groups were used in 4 experiments.

Experiment I. Vaginal smears were taken by lavage from 15 TP-treated rats when they were 62 days old and continued daily for 11 days. The smears were examined for types of cells and for the cell type which was present in the largest quantity. Four females, which had cornified cells for the first 6 successive days, were then placed overnight with adult males. Exposed females were autopsied 10 days post-exposure and number of concepti counted.

II. In 25 additional TP-treated rats, vaginal smears were taken for 14 days beginning on day 87. Vaginal smears were handled as described in Exp. I. Starting on the evening of the 100th day, each female was caged overnight with a different male for each of 10

* Persistent vaginal estrus is a condition characterized by the continuous presence of a predominance of cornified cells in the vagina.