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MAMMARY TUMOURS PRODUCED IN RATS BY THE ACTION OF ŒSTRONE TABLETS*

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THE relationship of œstrogens to the production of mammary cancer has been studied for the most part in mice of special cancer susceptible strains. In female mice of such strains it was found that a high percentage developed cancer spontaneously in the mammary gland. Animals which had raised litters showed a higher incidence of cancer than did those which were not mated. The possibility that œstrogens played an important rôle in the development of cancer was apparent many years ago but was established by various workers who found that removal of the ovaries prevented the development of breast cancer. Also it was noted that the earlier in life that ovariectomy was performed, the fewer the mice which developed cancer (Lathrop and Loeb;[1] Loeb;[2] Cori;[3] Murray[4]). More recently, however, Wooley, Fekete and Little[5] have found that even if female mice were spayed within 24 hours after birth, 26.8 per cent of them eventually showed mammary tumours. It was noted that the mammary glands and uterus were stimulated and it is suggested that in these animals adrenal gland secretion may have replaced œstrogen output from the ovaries. Male mice of cancer susceptible strains do not spontaneously develop mammary cancer, but œstrogen treatment of such animals stimulates the breast tissues, which eventually show malignant changes. (Lacassagne;[6] Burrows;[7] Bonser;[8] Gardner, Smith, Allen and Strong[9]). Because of the highly specialized genetic conditions essential for the production of mammary cancer by œstrogens in mice, and also because of the observations of Cook, Hieger, Kennaway and Mayneard[10] that various œstrogens were not carcinogenic when applied locally to the skin of mice (in contrast to the carcinogenic hydrocarbons), the rôle of the œstrogens as causative agents in mammary carcinoma has been considered chiefly from an experimental viewpoint.

More recently, however, evidence has been presented that œstrogen treatment may be followed by cancer of the mammary glands of rats, a species in which spontaneous cancer of the breast is rarely encountered. The occurrence of benign fibroadenoma, however, has been observed in rats and a special susceptible strain developed by Bryan, Klinck and Wolfe.[11] Recently they have found that these animals show associated changes suggestive of ovarian or pituitary dysfunction (Wolfe, Burack and Wright[12]). The effect of œstrogen on transplantable fibroadenoma has been studied by Emge,[13] who was unable to induce malignant changes. McEuen[14, 15] observed rats treated for prolonged periods with daily subcutaneous injection or oral feeding of œstrogen. Cancer, diagnosed by histological examination, was found in the mammary glands of 2 female rats treated daily with 30 μg. of œstrone after 533 and 624 days of treatment. No tumours of the breast were found after feeding 1,000 to 1,500 I.U. of crude œstrogen for prolonged periods (500 to 700 days). Astwood and Geschickter[16] observed an isolated case where mammary cancer developed in a spayed rat after œstrone injections. These results, while obtained in only a few animals, appeared suggestive when compared with control rats in which cancer of the mammary gland was not observed, and especially so when the apparently low incidence of spontaneous cancer of that organ in the rat was

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considered (reviewed by McEuen[14]). In 1939 Geschickter[17] reported the production of mammary cancer in 26 of 86 rats treated with œstrogen, and more recently[18] states the incidence to be "approximately 100 per cent of normally non-susceptible animals, males or females, castrates or non-castrates". Treatment of these animals consisted of injections or the implantation into the subcutaneous tissues of compressed pellets of œstrone or diethylstilbœstrol. The observations that œstrogens may cause mammary cancer, not only in susceptible strains of mice but also in rats where no genetic factor is essential, would appear of importance in a consideration of the etiology of cancer of the breast. The experiments to be presented were commenced in March, 1939, in an attempt to produce mammary cancer in rats by the subcutaneous implantation of œstrone tablets and to study the protective or curative action attributed to thyreotrophic extracts of the anterior pituitary gland by Cramer and Horning.[19] The latter aspect, however, will be treated in more detail in another communication.

METHODS

The rats used in the experiments were of the black-hooded strain maintained in the laboratory for the past 8 years, but whose genetic constitution is unknown. They were fed solely on Purina fox chow. Crystalline œstrone was made into small tablets, varying from 1 to 7 mg. in weight. These were made by great pressure and without any binding material, similar to those originally described by Deanesly and Parkes.[20, 21] The tablets were implanted either singly or in groups into the subcutaneous tissues of the back of female rats from 5 to 7 days old. The mammary glands were palpated at regular intervals and evidence of thickening or nodule formation was noted. Nodules when observed were measured and changes in size recorded. Examination of the gland by biopsy was made in some cases, but usually only after masses of approximately 1 cm. in diameter were palpable. The thyreotrophic extract used was prepared from hog whole pituitary gland, and represented the isoelectric precipitate described by Collip.[22] It contained 0.36 per cent total solids and injections of 0.04 c.c. twice daily for 5 days into 200 g. guinea pigs resulted in thyroid glands which averaged 69 mg. in weight. The rats received subcutaneous injections of this extract daily during the times indicated.

RESULTS

Mammary tumours.—The results were obtained from female rats which received the œstrone in tablet form implanted into the subcutaneous tissues of the back on the 5th, 6th and 7th day after birth. The total weight of œstrone used for individual rats varied from 4 to 11 mg. and was contained in from 1 to 3 tablets. At the end of 8 months the number of animals which survived and still retained palpable pellets was 47. One animal with a tumour had been killed after 226 days. In Table I the average time at which the tumours could be clearly established by palpation is recorded. At this time the size would be approximately 4 x 4 x 4 mm. but previous to this definite thickening of the mammary tissues and often small multiple lumps would be noted. These small tumours were hard and could be distinguished from the soft cystic masses also encountered. The time at which the tumours were first palpable is also included in Table I. Sections of these tumours were examined histologically and showed the microscopic picture to be described later.

TABLE I.
APPEARANCE OF PALPABLE TUMOURS IN FEMALE RATS AFTER ŒSTRONE TABLETS

Œstrone mg.	*No. of rats*	*Palpable tumours* *Earliest time days*	*Average time days*
4.0	8	263	335
4.5	3	226	286
5.0	5	263	300
6.0	7	271	334
6.0*	4*	275*	314*
6.5	2	341	343
7.0	3	263*	279
7.5	3	288*	309
9.0	1	271*	271
10.5	1	299*	299

* Multiple pellets.

From Table I it may be seen that with the range of doses of œstrone employed the total dose did not appreciably affect either the average time of appearance or the time of the first appearance of the tumours. Although in only a small number of cases has the weight of œstrone dissolved been calculated, it appears that with the larger doses approximately twice as much œstrone is absorbed than with the smaller. When multiple pellets were implanted the appearance of tumours was no more rapid than when single ones were used.

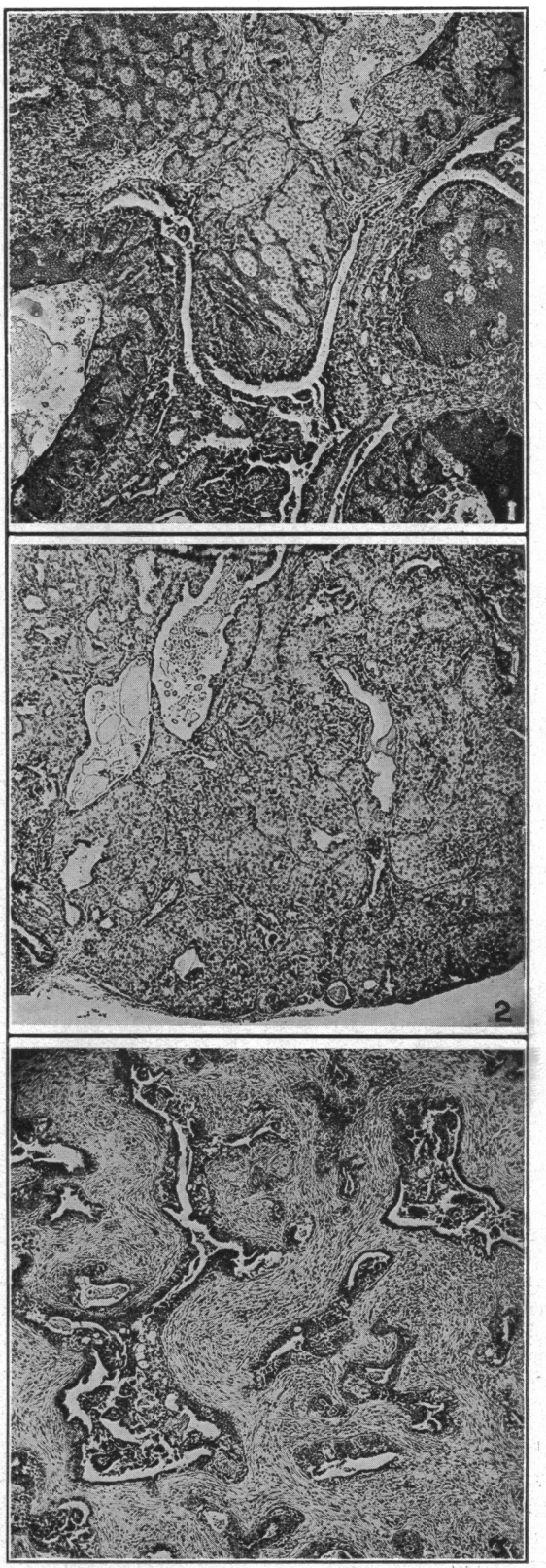
Incidence.—Up to the present time it has been found that 28 of the tumours show a histological

picture characterized by marked cellular hyperplasia. The incidence of such tumours, therefore, would appear to be approximately 60 per cent, but this figure will doubtless be raised as further tumours are examined microscopically. At present tumours are not subjected to biopsy until they have reached a diameter of at least 15 mm.

Effect of thyreotrophic hormone.—As previously mentioned, a number of the animals were treated with thyreotrophic hormone—each rat received 0.05 c.c., increasing to 0.25 c.c. by subcutaneous injection daily for 8 months after the pellets were inserted. In the treated group 17 out of 25 animals, or 68 per cent, have shown tumours, and in the non-injected control group 11 out of 19, or 58 per cent. Such treatment obviously had no effect on the incidence of the tumours described.

Gross characteristics of the tumours.—In most cases tumour formation occurred in more than one mammary gland of the rat simultaneously, but the individual size of the masses varied greatly. Tumours may occur in any gland of the animal and they frequently were found immediately under the nipple. Growth of the individual tumours was steady, but rather slow. To obtain a rough indication of growth the tumour size has been drawn every two weeks, and by measuring the greatest width and height of such drawings it was found that in 8 weeks' time tumours starting at 3 x 5 mm. had increased to 9 x 14 mm. These figures give an approximate indication of the average values obtained; great variation, however, was encountered and frequently new tumours appeared near the original, making accurate measurement difficult. The location of the tumours did not bear any relationship to the site of the œstrone tablet (located in the rat's back) and no growths of any kind were found adjacent to the pellets. Once tumours had reached a size of some 15 mm. diameter the animal was killed or used for subsequent experiments.

Histological appearance of the tumours.—The mammary glands in all the animals examined showed general evidence of stimulation. The glandular tissue was increased in amount and the ducts showed proliferative changes and were usually distended with secretion. In some cases large cystic areas were seen. The tumours were composed of more compact clearly defined areas of cells in which the epithelial components



Figs. 1, 2 and 3.—Mammary tumours in female rats following the administration of œstrone tablets.

showed an extremely marked hyperplasia. The masses of cells formed large rounded sharply demarcated solid areas, but in some a small central lumen was present into which occurred a shedding of the innermost cells. In most cases intense epithelial proliferation and squamous-cell metaplasia were present, but in some this was associated with an overgrowth of fibrous stroma. The general appearance of some of these tumours may be seen in the low-power microphotographs (Figs. 1, 2 and 3). The individual cells were seen to be of irregular shape and size and frequently showed mitotic figures. In all the specimens examined it has been found that despite the extreme hyperplasia the cells have neither exhibited any tendency to invade the stroma nor have they penetrated the well-defined basement membrane or adjacent tissues.

Absorption from tablets.—The tablets after removal from the rats were dissected free from fibrous tissue and weighed. Because of their small size it was found to be difficult to clean the tablets completely. The weights therefore are only approximate. In 19 animals the total weight of œstrone tablets implanted was 115 mg. After being in the rats for an average of 300 days their weight was 64.3 mg. In this time 50.7 mg. of œstrone had been dissolved, or roughly 44 per cent of the original tablets.

Body growth.—In these animals growth as reflected by body weight was only slightly affected at first but later was definitely retarded. The average weights of 41 rats may be compared with those of normal animals at various times after pellet implantation in Table II.

TABLE II.

AVERAGE BODY WEIGHT OF 41 FEMALE RATS AFTER 4-7 MG. TABLETS OF ŒSTRONE

	Age in weeks					
	1	6	12	18	24	30
Œstrone treated	12g.	104g.	132g.	143g.	155g.	161g.
Normal		102g.	171g.	192g.	198g.	209g.

Endocrine organs.—In the animals which have been killed up to the present it has been found that the weight of the pituitary gland was increased. The average weight of the pituitary gland in 15 animals was 28.4 mg. In one case (not included in the preceding average) a large adenoma was found weighing 380 mg. The histological picture showed the gland to be composed of chromophobe cells and was similar to that frequently described. The average weight of the adrenal glands from 10 animals was 37.1 mg., a value within normal limits. The ovaries were atrophic, weighing less than 10 mg., but the thyroid was of normal size and appeared normal on histological examination. The uterus showed a hyperplasia of the fibrous tissue and was lined with squamous epithelium. The Fallopian tubes were greatly hypertrophied. The squamous cells of the vagina were keratinized.

Other changes.—Within 2 months after insertion of the pellets 2 rats died, apparently from obstruction of the urinary passage—marked dilatation of the ureters and kidney pelvis was associated with infection. In 2 animals killed after 9 months the bladder was found to be distended with small stones. In two cases large ovarian abscesses were found. Lymphosarcoma which occurs spontaneously in this strain of rats (McEuen[23]) was encountered in two of the animals.

DISCUSSION

The changes, except those in the mammary glands, which have been described as occurring after the subcutaneous implantation of œstrone tablets in female rats, have been similar to those reported by numerous workers. In most cases previously published experiments have been of rather shorter duration and until recently the tablet method for the administration of œstrogens has not been employed. Enlargement of the pituitary gland has been noted by Zondek,[24] McEuen, Selye and Collip,[25] Deanesly,[26] Noble[27] and others, and the effects of œstrogens on growth of the rat have been studied by Spencer, Gustavson and D'Amour,[28] Zondek,[29] Deanesly,[26] Noble[27, 30] and others. Metaplasia of the uterine epithelium has been previously described by Selye, Collip and Thomson.[31] The rate of absorption of œstrogens administered in tablet form to rats has been estimated by Deanesly and Parkes[21] and Deanesly.[26]

Tumours of the mammary glands in rats have been consistently produced previously only by Geschickter.[17, 18] In his paper he has described cancer as occurring after 23 days of œstrogen treatment, diagnosis being by microscopic examination. In his series the mammary cancers have metastasized to the mediastinal lymph

nodes and lungs in several instances. In the experiments described above the histological appearance of the tumours seemed similar in all respects to that described by Geschickter, but metastases were not observed, possibly because a thorough enough search was not made, or because the experiment is not yet of long enough duration. Microscopic examination of the breast was not made during the early stages after œstrone treatment, but in 6 rats which died up to the 6th month after therapy commenced no evidence of cancer was found, whereas the first palpable tumour occurred after 226 days. Other workers also have previously failed to detect malignant changes following œstrogen pellet implantation in short term experiments (Deanesly;[26] Noble[27]). The times at which the tumours first appeared did not suggest that the dose of œstrone in the pellets or the number of pellets inserted influenced the speed of tumour formation.

At the present time further work is in progress to determine the action of other hormones on the mammary tissue under similar experimental conditions. The characteristics and biological properties of the tumours described are also being studied.

SUMMARY

Female rats from 5 to 7 days old received tablets of œstrone implanted into their subcutaneous tissue. The tablets varied from 1 to 7 mg. in weight and were used either singly or in groups. Following this method of treatment tumours were found in the mammary glands of 28 of 49 rats. The first tumour to appear was palpable after 226 days of treatment. The tumours were frequently multiple, slowly growing, and were not near the site of the œstrone tablet. Histologically the tumours showed extreme cellular hyperplasia but no evidence of invasion of the stroma or rupture of the basement membrane by tumour cells. They would appear to be essentially similar to those following œstrogen treatment in rats and described by Geschickter.

Other effects usually associated with prolonged œstrogen therapy have been noted. These included enlargement of the pituitary gland with adenoma formation, ovarian atrophy, uterine fibrosis with squamous cell metaplasia, and alteration in body growth.

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REFERENCES

1. LATHROP, A. E. C. AND LOEB, L.: *J. Cancer Res.*, 1916, **1**: 1.
2. LOEB, L.: *J. Med. Res.*, 1919, **40**: 477.
3. CORI, C. F.: *J. Exper. Med.*, 1927, **45**: 983.
4. MURRAY, W. S.: *J. Cancer Res.*, 1928, **12**: 18.
5. WOOLEY, G., FEKETE, E. AND LITTLE, C. C.: *Proc. Nat. Acad. Sc.*, 1939, **25**: 277.
6. LACASSAGNE, A.: *C. R. Acad. Sc.*, Paris, 1932, **195**: 630.
7. BURROWS, H.: *Am. J. Cancer*, 1935, **24**: 613.
8. BONSER, G. M.: *J. Path. Bact.*, 1935, **41**: 217.
9. GARDNER, W. V., SMITH, G. M., ALLEN, E. AND STRONG, L. C.: *Arch. Path.*, 1936, **21**: 265.
10. COOK, J. W., HIEGER, I., KENNAWAY, E. L. AND MAYNEARD, W. V.: *Proc. Roy. Soc. B.*, 1932, **111**: 455.
11. BRYAN, W. R., KLINCK, G. H. AND WOLFE, J. M.: *Am. J. Cancer*, 1938, **33**: 370.
12. WOLFE, J. M., BURACK, E. AND WRIGHT, A. W.: *Am. J. Cancer*, 1940, **38**: 383.
13. EMGE, L.: *Surg., Gyn. & Obst.*, 1939, **68**: 472.
14. MCEUEN, C. S.: *Am. J. Cancer*, 1938, **34**: 184.
15. *Idem*: *Am. J. Cancer*, 1939, **36**: 551.
16. ASTWOOD, E. B. AND GESCHICKTER, C. F.: *Arch. Surg.*, 1938, **36**: 672.
17. GESCHICKTER, C. F.: *Science*, 1939, **89**: 35.
18. *Idem*: *Radiology*, 1939, **33**: 439.
19. CRAMER, W. AND HORNING, E. S.: *Lancet*, 1938, **1**: 72.
20. DEANESLY, R. AND PARKES, A. S.: *Proc. Roy. Soc. B.*, 1937, **124**: 279.
21. *Idem*: *Lancet*, 1938, **2**: 606.
22. COLLIP, J. B.: The Cyclopedia of Medicine, Surgery and Specialties, Davis Co., Phila., 1939, p. 637.
23. MCEUEN, C. S.: *Am. J. Cancer*, 1939, **36**: 383.
24. ZONDEK, B.: *Lancet*, 1936, **1**: 776.
25. MCEUEN, C. S., SELYE, H. AND COLLIP, J. B.: *Lancet*, 1936, **1**: 775.
26. DEANESLY, R.: *J. Endocrinology*, 1939, **1**: 36.
27. NOBLE, R. L.: *J. Endocrinology*, 1939, **1**: 128, 216.
28. SPENCER, J., GUSTAVSON, R. G. AND D'AMOUR, F. E.: *Proc. Soc. Exp. Biol.*, N.Y., 1931, **28**: 500.
29. ZONDEK, B.: *Lancet*, 1936, **2**: 842.
30. NOBLE, R. L.: *Lancet*, 1938, **2**: 192.
31. SELYE, H., THOMSON, D. L. AND COLLIP, J. B.: *Nature*, 1935, **135**: 65.

SALICYLATE OF SODA FOR INFLUENZA.—Dr. J. T. Maclachlan (Dornoch) writes: As influenza appears to be prevalent just now I would like to draw attention to the value of salicylate of soda in its treatment. But a casual dose of aspirin or salicylate of soda is not sufficient. For the common type of influenza, beginning with headache, sharp rise of temperature, and severe myalgic pains, salicylate of soda should be given in the same way as it is given for rheumatic fever. In Scotland I found 20 grains for a man and 15 grains for a women, given every two hours for five or six doses, a reliable specific. Not more than 100 to 120 grains will be required for a man till the temperature is normal and the influenza gone, when tonics are required. Although I have treated hundreds of cases of influenza in a city practice I have seen very few cases of pneumonia. Hence I am forced to the conclusion that the salicylate of soda treatment is not only specific for the rheumatic type of influenza but actually prevents such complications as pneumonia, etc.—*Brit. M. J.*, 1940, **1**: 242.