Estrogen Production by Sertoli Cell Tumors of the Testis*

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There is a group of tumors of the testis in dog and man that cause feminization as a result of estrogen production. In this paper we shall demonstrate that these are neoplasms of the sustentacular cells of Sertoli which, in the dog at least, contain large amounts of estrogen.

Feminizing testicular tumors.—The syndrome of testicular tumors in dogs producing estrogenic effects was described by Greulich and Burford (13). The dogs are sexually attractive to other males (13, 33, 34), the tumors are often in cryptorchid testes (13, 19), the mammary glands are enlarged (13, 33), and generalized alopecia with increased pigmentation of the abdominal skin and swelling of the penile sheath are sometimes present. Squamous metaplasia of the epithelium of the prostate and posterior urethra is usual (11, 34).

The tumors often present similar cytologic characteristics. In the cases of Greulich and Burford they consisted of tubular structures of microscopic size. Innes (19) classified the growths as tubular adenomas (Sertoli cell tumors), and stated that some were of a strictly tubular variety while in others the tumor cells had transgressed the confines of the tubule to form irregular lobular masses. Zuckerman and McKeown (34) classified these neoplasms as adenocarcinoma, and considered that "essentially they represent a malignant development of all the cells of the seminiferous tubules, including the cells of Sertoli." The occurrence of tubular adenomas has been recognized, apart from the association with feminized males, for some time; thus Peyron (25) gave a histologic description of Sertoliform tumors of the testis in dogs more than 20 years ago.

Pick (26) described yellow nodular formations of cylindrical neoplastic cells arranged in tubules in the ovary and in the cryptorchid testis of man, which he called *adenoma tubulare ovarii testiculare;* the cells were about 15μ in height and consisted of clear cytoplasm with basal nuclei. Confirmatory studies of the pathology of these nodules by several workers have been reviewed by Krückmann (20).

Neoplasms of the human testis associated with growth of the breast have been reported frequently; commonest among them is the chorionic epithelioma (reviewed by Gilbert, 12). Three examples of testicular tumors with accompanying mammary hyperplasia have been interpreted as interstitial cell growths; the tumor cells were not arranged in tubular form. The case of Monaschkin (23) concerns a tumor of round, vacuolated neoplastic cells, which were stated to resemble the interstitial cells of Leydig. Hunt and Budd (4, 18) reported a man with symptoms of impotence and enlarged painful breasts, which subsided after removal of the associated testicular tumor. The growth was yellow and lobulated; the cells were ovoid in shape with eccentrically placed nuclei containing one or more large, basophilic nucleoli. Nation, Edmondson, and Hammack (24) described a man with mastopathy that did not disappear following removal of a testicular tumor. The cells of this neoplasm contained oval nuclei with one or more large, eccentric nucleoli; many fine droplets of fat were visible in the cytoplasm.

Estrogen production by the testis.—It is well known that the testis produces estrogen. Thus Fellner (11) observed that lipoid extracts of bull testis injected into castrate guinea pigs caused growth of the uterus. Qualitative studies indicating the presence of estrogen in this gland have been reported by others, also, for the bull (3, 8, 9, 21), and by still others for the deer (7). Zondek (32) found that the urine of stallions contained, on the average, 170,000 mouse units of estrogen per liter, while the urine of colts and geldings had about 0.3 per cent of this amount. Furthermore, the testis of the stallion contained 23,100 mouse units of estrogen in 350 grams, but less than 0.09 per cent of this amount was extracted from bull testis. From horse testes Beall (1) isolated α-estradiol (0.21 mgm. per kgm.) as its di-α-naphthoate, and estrone (0.36 mgm. per kgm.) as its 3,5-dinitrobenzoate; these are higher than the estrogenic values reported for any other tissue.

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METHODS

Five dogs with feminizing testicular tumors of spontaneous origin were available for study. Unless lactation was present, prolactin, 15 mgm. daily for 3 days, was injected subcutaneously. At autopsy the pituitary, thyroids, adrenals, testes, and prostate were weighed, and histologic preparations made of these tissues and of the breast. The tissues, and also normal testis from man and the dog, were stained for fat with Sudan, using frozen sections and the gelatin [1] technic of Heringa and tenBerge (14). Water and total fat content of the testes were determined as previously described (16).

Formalin-fixed testicular tumors of 2 dogs and, as a control, follicle-containing ovaries of 5 dogs were extracted for estrogen with fat solvents as follows: About 13 to 70 gm. of the tissues were minced, weighed, and extracted in a Soxhlet flask for 24 hours respectively with 95 per cent ethanol, ethyl ether, and again 95 per cent ethanol. The fat solvents were combined and evaporated to dryness. The residue was dissolved in ethyl ether, washed 4 times with distilled water, and evaporated to dryness. The residue was again taken up in 95 per cent ethanol, diluted with water to reduce the concentration of alcohol to 70 per cent, and extracted twice with petroleum ether in a separatory funnel; petroleum ether was discarded and the ethanol fraction evaporated to dryness. The residue was dissolved in sesame oil.

Bioassay was done on immature female albino mice, 21 days of age and weighing 6 to 8 gm., by the method of Evans, Varney, and Koch (10). Five mice were used for each assay level, and 10 for the controls.

RESULTS

Anatomic status.—The animals were stray dogs of mongrel type and weighed between 9.3 and 13.6 kgm. The ages were unknown, but 4 of the animals were old since they had dense cataracts and worn incisors; 1 dog with bilaterally cryptorchid testes was considerably younger, for it had normal eyes and teeth.

The dogs were sexually attractive to other males. All had hypertrophy of the mammary papillae (Fig. 1); 1 dog lactated spontaneously, 2 lactated after prolactin injections, and in 2 dogs lactation was not induced. In the dogs that lactated 0.5 to 1.0 cc. of lipid-rich milk was expressed from each of the papillae.

The prostate gland weighed from 6 to 38 gm. Squamous metaplasia of the prostatic epithelium was present in all cases. In the smallest gland the acini were atrophic, and the keratinized epithelium characteristic of estrogen dominance was present only in the prostatic urethra; in the other glands metaplasia was present throughout the acini also.

Constant changes were not observed in the suprarenal glands; the weight of a single gland varied from 0.334 to 2.0 gm. In 2 dogs the suprarenals were small and there was atrophy of the cortex with "brown degeneration," a lipoid band in the cortex encircling the medulla as described by Cramer and Horning (5, 6) in certain strains of mice. In 2 dogs the suprarenals were normal and in 1, whose suprarenals weighed 1.48 and 2.0 gm., small cortical adenomas were present. The pituitaries weighed from 35 to 73.5 mgm.; neither they nor the thyroids were remarkable. One of the dogs had a squamous carcinoma of the anus with retroperitoneal metastasis in lymph nodes.

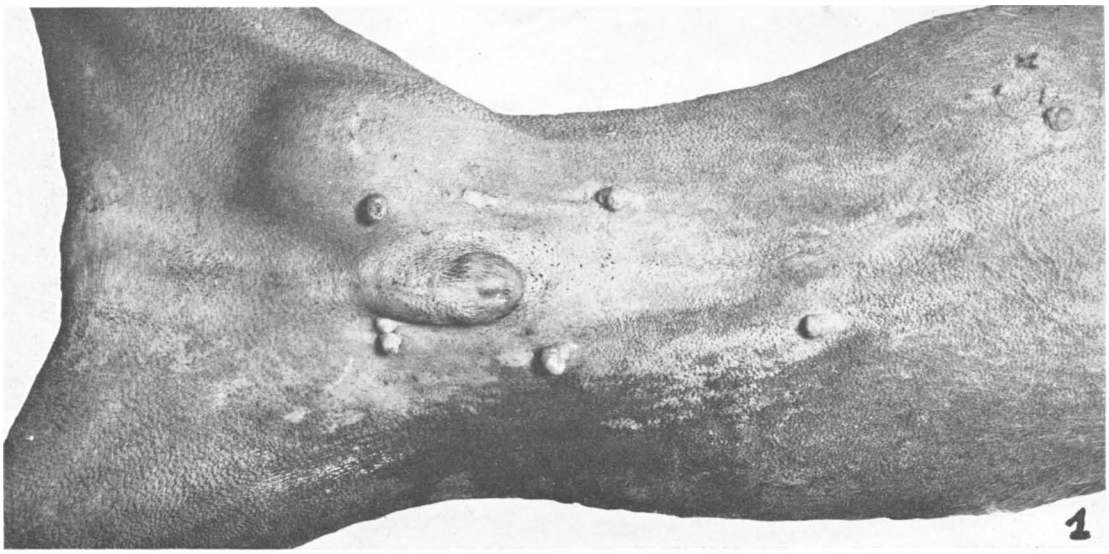
The testicular tumors were 7 in number, unilateral in 3 dogs and bilateral in 2; 3 of the growths were in cryptorchid testes, bilateral in 1 animal. Metastases were not present. The nontumorous testicular tissue showed moderate to severe atrophy of the germinal epithelium in all the dogs. The feminizing neoplasms of the dog testis correspond to the lobulated tumors with high fat content of Huggins and Eichelberger (16); in the gross they are firm, white in color, and are bossed in contradistinction to the soft orange-yellow interstitial cell tumors, which usually occur as single discrete masses.

In 3 of the growths the cells were arranged in tubules; in 2 the cells were diffuse (Fig. 2) and tubular arrangement was not apparent, while in 2 others poorly formed, distorted tubules were observed. In the tubular types there were many cells lying free in the lumina; the nuclei were basal in cells at the periphery of the tubules. The nuclei in the tubular types contained much chromatin, which was not so extensive in the diffuse types. In all cases large nucleoli were prominent in the cells.

The tumor lipids.—In normal adult dog and human testis stained for sudanophilic material (fat), large quantities of lipid were found in the interstitial cells of Leydig and in the Sertoli cells. The germinal epithelium was practically fat-free. All the estrogen-producing tumors contained intracellular sudanophilic material, usually in a single large globule; in the cells of interstitial cell tumors it was distributed diffusely in many small droplets. The total lipid content of the tumors was 35.7 to 75.1 gm. per kgm. of fresh tissue; the average value for normal canine testis is 20.3 gm. per kgm. (16).

Tumor estrogens.—Two of the testicular tumors from feminized dogs were extracted for estrogen and values of 3.4 and 70 μgm. per kgm. expressed in terms

[1] We are indebted to Professor P. P. H. De Bruyn for assistance in the preparation of the gelatin sections, and to Dr. William W. Scott for aid in the estrogen assays. Dr. Erwin Schwenk, of Schering Corporation, generously furnished prolactin and estradiol.



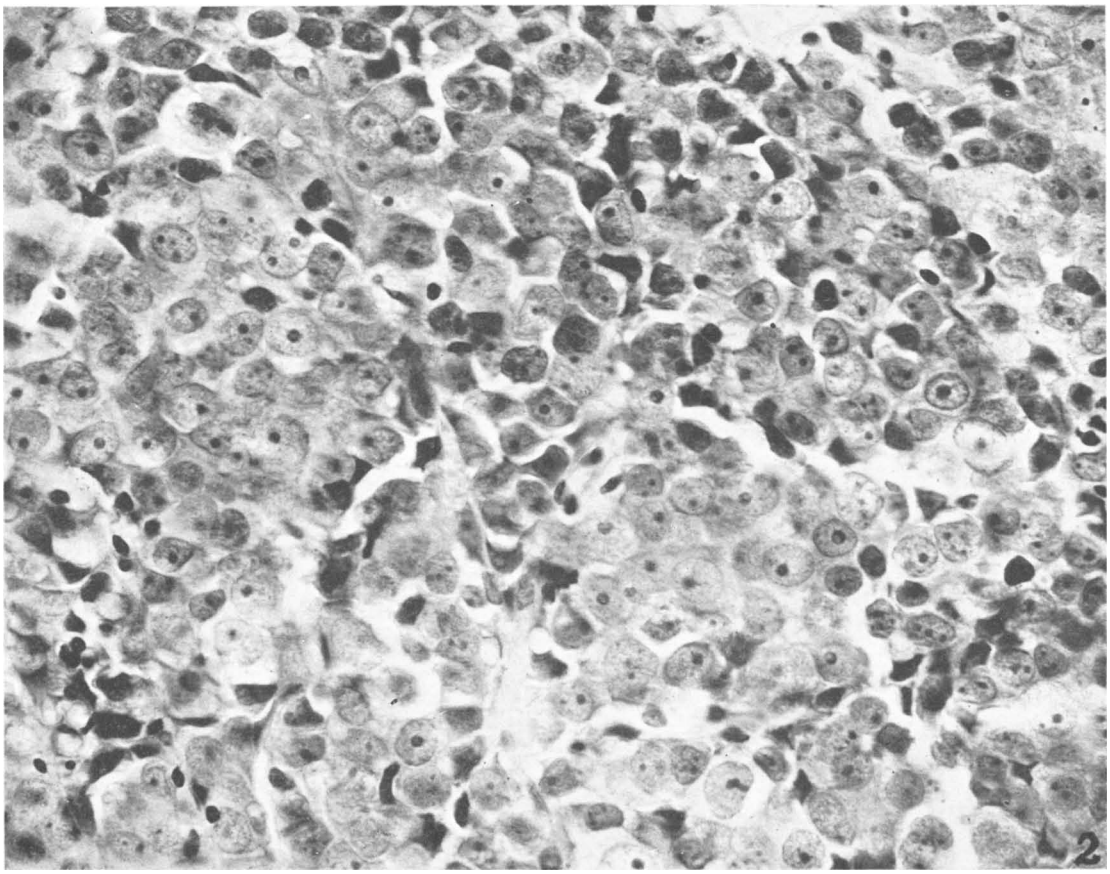


FIG. 1.—Hypertrophy of mammary papillae in a dog with a Sertoli cell tumor in a cryptorchid testis.
FIG. 2.—Sertoli tumor of diffuse type from the testis of a feminized dog. Mag. × 475.

of α-estradiol benzoate, were obtained. Estrogen could not be extracted from a Sertoli cell tumor that had not caused feminization. Combined ovaries weighing 13.7 gm. from 5 dogs at estrus (vaginal bleeding) contained the estrogen equivalent of 34 μgm. per kgm.

DISCUSSION

The site of estrogen production in the testis has not been identified previously. The germinal epithelium of the gland is certainly not its source: The tumors derived from germinal epithelium (seminomas) do not cause squamous metaplasia of the prostate (17), and femaleness occurred in a male dog with bilateral cryptorchism with associated testicular tumors in the present series. While it is readily feasible to eliminate the germinal cells by cryptorchism or administration of certain steroids (androgen or estrogen) no experimental approach has been devised to eliminate selectively the interstitial apparatus or the Sertoli cells. The overgrowth of a specialized cell in a tumorous process provides an opportunity to study the physiologic effect of overgrowth of a single cellular type.

Evidence advanced previously regarding the testicular origin of estrogen is inconclusive; both Sertoli and Leydig cells have been implicated, but the evidence has been inferential rather than substantive.

In a human hermaphrodite with breast hypertrophy Witschi and Mengert (31) found an abundance of Sertoli cells in abdominal testes, to which they ascribed the production of estrogen; estrogen was excreted in the urine and it disappeared after castration. In this testis clusters of the interstitial cells of Leydig were present, though they were small and did "not give the impression of much activity." These authors point out the morphologic homology of ovarian granulosa and sustentacular cells of the testis, stating that both arise from the follicle cells of the primordial gonia.

Evidence that the Leydig cell produces estrogen is derived from the assumption that certain tumors of the human testis associated with mammary hyperplasia are interstitial cell growths (18, 23, 24). This interpretation does not fit in with other cases where interstitial cell neoplasms produced large amounts of the male sex hormones. In children several tumors of the testes that cytologically resembled interstitial cell growths of the dog have been described (27, 29); they effected precocious growth of the sexual organs and muscular development—clear evidence of androgen production. An adult described by Masson (22) with malignant interstitial cell carcinoma had an extremely high titer of urinary androgen but no gynecomastia; extracts of the urine of this man contained 980 to 1,040 mgm. of 17-ketosteroids *per diem* (30) in the ketonic fraction.

It is now possible to prove that the estrogen-producing tumors of the testis are derived from Sertoli cells; the evidence is based on histochemical study of the lipids and on estrogen extraction and assay.

Sertoli (28), in his description of the sustentacular cells of the tubules of the testis, stated that they contain fat. By histological methods the lipids (11) of the testis were found present normally in 2 locations: the sustentacular and the interstitial cells. The germinal epithelium is nearly fat-free.

All the testicular tumors of feminized dogs contained large amounts of lipid; from 75 to 370 per cent more fat than normal canine testis. In 3 the neoplastic cells were arranged in tubular formation while in 2 others tubular formation was present, but the tubules were abortive and transition stages were traced between strictly tubular and diffuse patterns of the same growth. By definition the cells of the testicular tubules with high lipid content are sustentacular cells.

The estrogen content of these lipid-rich neoplasms is considerable; in 1 case it was equivalent on bioassay to 0.07 mgm. per kgm., calculated as α-estradiol benzoate, being twice the content of the ovaries at estrus and one-third of the amount in equine testis, the richest known source of estrogen.

Not all the Sertoli cell tumors of the testis produce estrogen in sufficient quantity to exert a physiological effect. With respect to the prostate of the castrate dog, androgen in adequate amount is able to mask the action of injected estrogen; specifically testosterone propionate, 10 mgm., is able to mask the effects of diethylstilbestrol in amounts up to 0.4 mgm. (15). Therefore unless the amount of androgen produced is exceeded by estrogen in fixed ratio the feminizing effects of estrogen on the prostate are concealed.

Do the Leydig cells produce estrogen? Since the observations of Bouin and Ancel (2) most workers have agreed that androgen is produced in the interstitial cells of the testis. It would be surprising if physiologically antagonistic substances such as androgen and estrogen were produced in the same cell. A close cytologic similarity exists between the diffuse Sertoli cell tumors of the canine testis (Fig. 2) and those human tumors associated with mammary growth and previously classified as interstitial cell tumors. The interstitial cell growths of the dog are characteristic in their extratubular location and highly vacuolated appearance; invariably they are associated with tall cylindrical prostatic epithelium, often hyperplastic and cystic (evidence of androgen dominance), and squamous metaplasia is lacking. The cytologic appearance is quite different from the diffuse Sertoliform tumors, where diffuse vacuolation is not observed.

Difficulty exists concerning nomenclature of the feminizing neoplasms of the testis: the term tubular

adenoma is unsatisfactory, since in certain cases the tubules are vestigial or absent; for the same reason the term adenocarcinoma (34) is inexact, and none of these tumors, furthermore, has been found to metastasize in dog or man. These difficulties may be eliminated by the use of the terms tubular or diffuse Sertoli cell tumor.

CONCLUSIONS

Feminizing tumors of the testis are rich in lipids and are growths of the sustentacular cells of Sertoli; tubular and diffuse types and intergrades between these kinds are described. Their estrogen content is considerable; in one case, equivalent to 0.07 mgm. of α-estradiol benzoate per kgm. of fresh tissue—an amount twice as great as in ovaries containing large follicles at estrus.

The cells of Sertoli in the testicular tubules produce estrogen.

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