Role of Gonadotrophic Hormone in the Initiation and Progression of Adrenal Tumors in Ovariectomized Mice*

H. S. TULLOS,† A. KIRSCHBAUM,‡ and J. J. TRENTIN

(Departments of Anatomy and Surgery, Baylor University College of Medicine, Houston, Texas)

SUMMARY

Ovariectomy of (C57 × NH) F1 mice resulted in estrogen-secreting adrenal adenomas and estrous-type vaginal smears. Parabiosis with intact (C57 × NH) F1 females usually converted the vaginal smears from estrous to diestrous but produced a constant-estrous type smear in the intact parabiont. Ovariectomy of the latter resulted usually in restoration of an estrous-type smear in the tumorous animal and in its own smear's becoming diestrous. This is interpreted as indicating preferential action of the high gonadotrophic hormone levels of the tumorous animal on, or utilization by, the ovaries of the intact parabiont. The consequent diminution of estrogen output by the adrenal tumors is interpreted as evidence for an etiological role of the high postcastration gonadotrophic hormone levels in the induction of estrogen-secreting adrenal adenomas. Ovariectomy of strain CE mice resulted in estrogen-secreting adrenal adenoma formation within 6 months, with progression to adrenal carcinomas by 18 months. Progression was prevented by introduction of ovarian isografts at 6 months postcastration, indicating an etiologic role of the high postcastration levels of gonadotrophic hormone in the progression of adrenal adenomas to carcinomas.

In a variety of animal species (4, 22) functional adrenal-cortical adenomas and carcinomas will develop following surgical (12) or "physiological" castration (15). Tumors may be prevented by hypophysectomy (6) or by estrogen (26) or androgen (34) injection. Prolonged administration of adrenocorticotropic hormone will not induce adrenal neoplasia (16), and continued postcastrational injection of deoxycorticosterone acetate results only in a brief delay in the time of tumor appearance (11). Doses of cortisone which caused adrenal cortical atrophy did not prevent the development of adenomas in ovariectomized mice (17, 18).

Prolonged parabiosis of intact females with castrated females results in ovarian enlargement and, ultimately, neoplasia (21). Castrated mice bearing a splenic ovarian graft develop ovarian tumors if splenic abdominal adhesions do not occur (2), but develop a greatly reduced number of adrenal tumors (8, 9, 13).

Following castration there is an increased pituitary gonadotrophin content (10, 23), and this is generally regarded as being responsible for the ovarian tumors. The possibility that it is also related to the induction of postcastrational adrenal adenomas has also been considered (5, 12, 20). Hypophysectomy of rats bearing ovariectomized-adenoma-induced estrogen-secreting adrenal adenomas results in suppression of estrogen secretion, which is restored by injection of gonadotrophin but not by injection of adrenocorticotropic (12).

Monsen and Kirschbaum have noted that, if castrated mice bearing estrogen-secreting adrenal tumors are parabiosed with isologous intact females, the vaginal cytology of the tumor host becomes diestrous while the previously cyclic female tends to go into constant estrus. They suggest that gonadotrophin may be the stimulus for both the
ovarian and the adrenal tumors but that its site of first preference is the ovary (14, 20). Experiment I is an attempt to confirm this observation.

Experiment II is an attempt to determine the role of gonadotrophic hormone in the progression of adrenal adenomas to carcinomas, that occurs in castrated mice of some strains, such as the CE strain (25).

MATERIALS AND METHODS

Experiment I: Role of gonadotrophic hormone in the initiation of adrenal adenomas.—Nine (C57 X NIH)F1 hybrid female mice were castrated at 6 weeks of age. By 10 months postcastration the existence of adrenal adenomas was indicated by a continuous proestrous-estrous type vaginal smear. These animals were then each parabiosed with a 4- to 6-week-old intact isologous female. The incision was from foreleg to hindleg, and the peritoneal cavities were opened and joined. Vaginal smears were made, usually at 2-day intervals. Forty days after parabiosis, five of the nine intact parabionts were castrated and both parabionts followed by vaginal smears for another 30 days.

Experiment II: Role of gonadotrophic hormone in the progression of adrenal adenomas to carcinomas. Forty-eight female CE mice were castrated at ages of 100-175 days. These animals were subdivided into control and experimental groups, matched as to age, litter derivation, and age at castration. The 24 untreated control mice were autopsied at 18 months postcastration. Single sections were taken of the submandibular gland and the uterus, and serial sections of both adrenal glands. The vaginal smears of all 24 experimental mice showed evidence of estrogen production by 6 months postcastration, presumably of adrenal origin. The presence of adrenal tumors was confirmed by laparotomy, and bilateral subcutaneous ovarian isografts were made. The animals were autopsied at 18 months following castration and the tissues removed for histological examination.

RESULTS

Experiment I.—Subjective estimates of the stage of the estrous cycle of each member of the nine pairs of parabionts were made by examination of the types of cells in the vaginal smears (Charts 1 and 2). Prior to parabiosis the smears of tumor-bearing mice indicated a constant proestrous estrogen level which, following parabiosis, changed to a diestrous level. Simultaneously, the smears of the intact parabiont tended to become estrous in type. Upon castration of the intact parabiont, its vaginal cytology changed to that of a diestrous state, whereas the tumor-bearing parabiont returned to its original proestrous-estrous type smear.

These data, though generally confirmatory of the results of Monsen and Kirschbaum (19, 20), are not absolutely uniform. In pairs III, V, and VI, the intact female continued to show erratic cycles. In pairs III, V, and IX, the tumor host became diestrous but did not remain persistently so. Since 2 per cent of injected estrogen crosses the parabiotic “barrier” in rats (1), the explanation may lie in a “leakage” between the two parabionts. At autopsy, bilateral adrenal-cortical alpha-cell tumors were still present in all nine of the tumor-bearing mice. The adrenal cortex appeared histologically normal in all nine of their intact and castrated parabionts. No ovarian tumors were noted.

Experiment II.—Fourteen of the 24 castrated control mice survived 18 months after castration. At autopsy, kidney-sized adrenal carcinomas were present bilaterally in nine mice, unilaterally in the remaining five. Microscopically, “alpha cell” proliferation predominated, but rosette-like areas of clear “beta cells” could be found (25). All animals within the control group bore evidence of androgenic stimulation as manifested by submandibular gland histology.

Fifteen of 24 mice receiving ovarian fragment grafts survived an additional 12 months after grafting, or 18 months after castration. At autopsy there was no gross evidence of adrenal carcinoma formation. Histologically, the adenals revealed only subcapsular “alpha cell” adenomas similar to those present in four animals killed 6 months postcastration. Ovarian tumors of the granulosa-cell type appeared in the ovarian grafts of three animals. Grossly, the ovarian tumors were kidney-sized—bilateral in one animal, unilateral in the remaining two. It is postulated that the ovaries grafted into mice bearing adrenal adenomas supplied sufficient estrogen to inhibit pituitary gonadotrophic hormone secretion and thereby abolished the stimulus for progression from adenoma to carcinoma.

DISCUSSION

Adrenal adenomas arise postcastrationally (12, 15), are inhibited by estrogen (26) or androgen (34), but fail to respond to adrenocorticotropic hormone (16) or to deoxycorticosterone (11). Hypophysectomy is effective in preventing their development (6). If ovaries are transplanted to the spleens of ovariectomized mice, ovarian tumors occur in high incidence, but the adrenal tumor incidence is greatly reduced in these “physiologically castrated” animals (8, 9, 13). When ovariecto-
mized mice bearing functioning adrenal tumors are parabiosed with young untreated females, estrogen secretion by adrenal tumors is diminished, and simultaneously ovarian hyperfunction appears in the parabiont, as manifested by the vaginal responses of both parabionts. It is postulated, therefore, that gonadotrophic hormone is the agent responsible for the initiation and progression of adrenal cortical neoplasms in mice but that ovarian tissue is the target organ of preferential action or preferential utilization of gonadotrophic hormone; in the absence of this target the hormone may act unrestrainedly on the adrenal gland to induce estrogen secretion and neoplasia. Transplantation of ovaries into CE mice bearing ovarioectomy-induced adrenal adenomas did not cause regression of the adenomas but did prevent their progression to carcinoma. The capacity of functioning ovarian grafts to inhibit this adenoma-carcinoma sequence is apparently due to ovarian estrogen production, with its subsequent inhibition of gonadotrophic hormone. Once formed, these carcinomas may be relatively independent of elevated gonadotrophin, for they transplant to intact

**Chart 1.**—Vaginal cytology of (C57 × NIH)F1 hybrid ovarioectomy-adrenal-tumor-bearing and intact female mice before and after parabiosis and subsequent castration of intact female.
isolologous hosts (3). The progression of endocrine tumors through successive stages of dependence and autonomy has been extensively studied and reviewed by Furth (7).

It is of interest that, whereas ovarian estrogen is effective in the prevention of carcinoma formation, adrenal estrogen is not. The adenomas which develop postcastrationally function to produce estrogen titers sufficient to partially cornify the vaginal epithelium, yet, if untreated, go on to form carcinomas in susceptible mouse strains. The difference between these two estrogens may be only that of dosage or may represent a qualitative dif-

Chart 2.—Vaginal cytology of (C57 X NH)F1 hybrid ovariectomized-adrenal-tumor-bearing and intact female mice before and after parabiosis. Castration of the intact parabiont was not performed in these pairs.

REFERENCES


Role of Gonadotrophic Hormone in the Initiation and Progression of Adrenal Tumors in Ovariectomized Mice

H. S. Tullos, A. Kirschbaum and J. J. Trentin