Histological Study of Adrenal Cortical Tumors in Gonadectomized Mice of the ce Strain*†

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It has recently been reported from this laboratory that carcinoma of the adrenal cortex occurred in a high percentage of cases in extreme dilution strain ce mice that were gonadectomized at 2 days of age (3). The material collected from these animals is especially valuable because it gives a chance to study and detect the very early changes that precede and lead to the formation of rare tumors of this type. Therefore, in this histopathological study special emphasis will be given to the description of these early changes, which are unique in this strain and may justifiably be considered as significant steps leading to tumor formation.

MATERIAL AND METHOD

A total number of 131 gonadectomized mice were observed, 70 spayed females and 61 castrated males. Gonadectomy was performed at 2 days of age. The method of removing the ovaries has been described previously (1, 2). The testes were approached through a small midventral incision, and the epididymides and a short part of each vas deferens were also removed.

Groups of experimental animals were killed at monthly intervals, and in every case a careful search was made for gonadal regeneration. In the earlier age groups both adrenals were serially sectioned; in the older age groups when the adrenal tumors were very large serial sectioning seemed unnecessary and only a few sections were cut.

Twenty normal virgin females and 23 males were used as controls. Both adrenals of all these animals were serially sectioned. No adrenal tumors occurred in any of them.

Observation on the Adrenals of Spayed Females

At 4 months.—The earliest abnormal areas, which were unique in the ce strain of mice, were found in the adrenals of animals killed at 4 months of age. The significance of these areas became evident only because one was able to observe what followed them rather than the way they were at this stage.

They were composed of a small group of cells in follicular or arch-like arrangement, which occasionally contained a lumen, and were situated immediately below the connective tissue capsule. The cytoplasm of these cells formed a syncytial protoplasmic network, in some cases filling the lumen. The nuclei that were scattered throughout this network were oval in shape, contained very finely distributed chromatin granules, and usually one prominent compound nucleolus. The areas were surrounded by circularly arranged connective tissue fibers (Fig. 1). The whole structure resembled somewhat the seminiferous tubules of a cryptorchid testis in which only Sertoli cells are present in a protoplasmic syncytium.

The adrenals of 3 out of 4 mice belonging to this age group contained such areas, and in each of 2 glands 2 were present.

At 5 months.—Only 1 animal was killed at this age and a similar abnormal area was present in one adrenal.

At 6 months.—Five animals represented this age group. The next series of changes was perhaps best represented by one adrenal of mouse 2088. There were now several groups of round areas in close proximity, composed of cells in syncytial arrangement, and their presence caused a slight elevation on the surface of the adrenal. At the base of some of these areas large polyhedral cells were present arranged in a stratified layer. They had large oval nuclei containing chromatin granules of medium size and several nucleoli. A small amount of slightly basophilic cytoplasm surrounded each nucleus. These cells infiltrated about half way into the fascicular zone and destroyed its cell components. Similar cells formed the bulk of the large tumors that occurred in the adrenals of the older animals. The fact that at their early appearance these cells were in close association with areas composed of syncytial cells perhaps justifies the postulate that the latter areas were precancerous lesions.

The other adrenal of this animal (2088) contained...
a small precancerous area, which caused a slight bulging of the capsule.

In one adrenal of each of 2 other animals (2086 and 2175) small cancerous areas were present in irregular nodular arrangement. In the fourth animal of this group the neoplastic area in one adrenal was considerably larger, projecting about half way into the medulla. The tumor consisted entirely of large polyhedral cells and showed many mitotic figures. It was not encapsulated, although the surrounding cell columns and sinusoids, which were compressed by the expanding growth at some places, gave this impression. The other adrenal did not show precancerous or cancerous changes.

Both adrenals of the fifth mouse in this group were normal.

At 7 months.—Four animals were killed at 7 months of age. In one adrenal of mouse 2252 the neoplastic area involved the whole depth of the cortex at that focus; in the other, both precancerous and cancerous areas were present in close proximity. Enlarged cells, some in mitotic division, were present between the fibers of the capsule, causing a slight elevation of the gland surface.

One adrenal of mouse 2259 was normal. In the other, which was somewhat enlarged, the cancerous area occupied more than half of the gland. In this, as in all the larger tumors, the nuclei were more pleomorphic, the cytoplasm was slightly basophilic and small in amount, and the cell outlines were indistinct. Mitotic figures were numerous. A rich network of capillaries provided a good blood supply. The stroma was very scanty.

Small carcinomatous areas were present unilaterally in the adrenals of mice 2260 and 2250 while the opposite glands were normal.

At 8 months.—One animal was killed in this age group (2257). Both adrenals were carcinomatous, and one of them was greatly enlarged.

At 9 months.—Two mice represented this age group (2686 and 2689). In 1 a tumor of medium size was present in one adrenal, while the opposite gland was tumor-free. In the other animal (2689) one adrenal was considerably enlarged by tumor. In the opposite gland a small tumor was present near the capsule.

In the adrenals of both these animals a new type of tumor cell occurred, which seemed to have originated between the fibers of the capsule. These cells were small, cuboidal in shape, and arranged in rows. They had large, dark-staining, oval nuclei with evenly distributed, coarse chromatin granules. A small amount of slightly basophilic cytoplasm surrounded each nucleus. These cells resembled the follicular cells of young ovarian follicles.

Older Age Groups

From this age on, description of the tumors of individual age groups seems unnecessary. The cells already described were present in all, differing only in proportion. In almost all instances the second (cuboidal) cell type occurred only when a tumor of considerable size formed by the first (polyhedral) cell type was already present. However, there were a few exceptions. In one adrenal of mouse 2273 (11 months) rows of cuboidal cells were present between the fibers of the capsule at a wedge-shaped area, which caused a slight elevation of the gland surface. At the inner aspect of this a small follicle-like area was present in which the central part was filled with nuclei scattered in a protoplasmic network (precancerous area), while at the periphery polyhedral cells were present in irregular arrangement. In the other adrenal a medium-sized tumor composed of the polyhedral type of cell was present.

In most of the large tumors the bulk of the tissue was formed by diffusely arranged polyhedral cells which, judging from the number of mitotic figures, multiplied more rapidly (Fig. 2). The cuboidal cell type was usually arranged in rows and cords showing some degree of attempted gland formation (Fig. 3). Although usually these cells were more prominent near the periphery, they were also found intermingled with the diffusely arranged polyhedral cells. Scattered among both types of tumor cells, giant cells possessing yellow pigmented cytoplasm and often multiple nuclei were found in many tumors in varying numbers. Blood vessels were numerous and consisted mainly of capillaries and endothelium-lined spaces between groups of tumor cells. In smaller carcinomas the stroma was very scanty, forming only a delicate framework (Fig. 4). After the capsule of the gland was invaded, larger trabeculae projected into some of the larger tumors, dividing them into irregular lobules (Fig. 5).

In some cases the syncytial type of cell, which was described as forming precancerous areas, was present near the periphery of cancerous areas (Fig. 4). In one large neoplasm this cell type evidently persisted and formed a considerable part of the tumor. The syncytial cytoplasm of these cells contained lipid globules (Fig. 6).

Many of the large tumors had broken through the adrenal capsule; however, this was usually present around a small part of the gland. Beneath it remnants of normal cortical tissue and narrow compressed zones of medullary cell cords were always present. In the large tumors the central areas often showed hemorrhage, necrosis, and occasionally calcification.

While the normal size of a mouse adrenal is about
1 × 2 mm. in diameter, several of the cancerous glands reached 15 × 20 mm. and were easily detectable in the live animal by palpation.

Two animals that showed attempted ovarian regeneration should be mentioned individually. Mouse 1147 (killed at 13 months) showed at autopsy a dense nodule in the adipose tissue of the mesosalpinx and microscopic examination gave proof of the presence of ovarian tissue. Several primary and anovular follicles, areas of luteinized cells, and small cysts lined by ciliated columnar epithelium were present. The bulk of the nodule was composed of atypical cells forming an ovarian carcinoma. This mouse also had a large adrenal cortical carcinoma. Structurally the ovarian and the adrenal carcinomas were strikingly similar. A medium-sized tumor was found in the ovarian region of mouse 2418 (killed at 15 months). Microscopic sections did not reveal any normal ovarian tissue and there was only gross evidence of an ovarian origin. This tumor also resembled structurally the adrenal cortical carcinomas.

An interesting anatomical difference existed between other strains and the ce strain concerning the position of the adrenal glands. While in other strains there is a distance of about 2 mm. between the inner aspect of the cephalic pole of the kidneys and the adrenals, in the ce strain the surfaces of these 2 organs are in direct proximity. In spite of this the kidney was never invaded by the adrenal tumors.

Metastases to the lungs were observed in microscopic sections in 8 instances. As the lungs were sectioned only when suspicious nodules were noted upon gross examination some of the smaller metastases may well have escaped detection.

To summarize the changes that seemed to occur in 3 main steps: (a) The first step was the appearance of small groups of syncytial cells near the capsule, characterized by oval nuclei scattered in a protoplasmic network. The nucleus contained very fine chromatin granules and usually one prominent compound nucleolus. These areas formed round follicles, which occasionally contained a lumen and were surrounded by circularly arranged connective tissue fibers. Since in later abnormal development these areas played a prominent part they were considered as precancerous regions. They occurred first in the adrenals of 4 month old animals, but were also found later at the periphery of already cancerous areas. (b) The second step was the appearance of atypical polyhedral cells, first at the base of the follicles that formed the precancerous regions. The nuclei at first were mostly oval—later pleomorphic, and contained medium-sized chromatin granules and several nucleoli. The cytoplasm was slightly basophilic and small in amount, there was a lack of polarity, and from their earliest appearance these cells showed unusual mitotic activity. In diffuse masses they projected first toward the center of the gland and later outward, breaking through the capsule. (c) The third step was the appearance of rows of cells that seemed to originate between the fibers of the adrenal capsule in animals in which tumors formed by polyhedral cells were already present. These cells were cuboidal in shape, and had oval dark-staining nuclei that contained coarse chromatin granules. They were arranged in parallel rows or cords showing an attempted gland formation. They usually occupied a peripheral position, but in the larger tumors they were also found intermingled with polyhedral cells. They were rarely seen in the process of mitotic division.

Observations on the Adrenals of Castrated Males

At 4 months.—The adrenals of 3 animals were examined at this age. In one adrenal of mouse 2082 a small round area was present, composed of cells in syncytial arrangement. The cells were similar to those found in the adrenals of several of the ovariectomized females of this age group.

At 5 months.—One animal was examined at this age. In one adrenal a small, completely encapsulated, benign tumor was present immediately below the capsule. It was composed of polygonal cells with large, round nuclei surrounded by small amount of cytoplasm.

At 6 months.—Five animals were sacrificed at this age; none of the adrenals contained tumors. Small areas composed of cells in syncytial arrangement were present below the capsule in the adrenals of 2 mice unilaterally.

At 7 months.—Two mice were killed at this age. Unilateral cortical tumors were present in 2 of them. In both adrenals the neoplastic areas were narrow, but extended from the capsule to the medulla. The nuclei of the tumor cells were mostly oval, though showing some variation in shape and size, and contained finely distributed chromatin granules and sev-
eral nucleoli; the cytoplasm was scanty and slightly basophilic. The cells were polygonal, showed a lack of polarity, and several were in the process of mitotic division. The tumors were not encapsulated and were considered carcinomas; one was surrounded by an area of hypertrophied cells and a slight elevation of the gland surface was evident.

At 8 months.—One animal with a small cortical carcinoma in one adrenal represented this age group.

At 9 months.—Five mice were killed at this age. In 2 animals cortical carcinomas were present unilaterally, and in 1 bilaterally. In mouse 2693 about half of the gland had been destroyed and replaced by tumor cells. The carcinomatous adrenal of mouse 2263 was about 3 times as large as the normal gland. One adrenal of mouse 2315 was somewhat enlarged by the carcinoma, while the opposite gland contained a very small tumor. All these neoplastic areas were composed of large, irregularly arranged, polyhedral cells similar to those that formed the tumors of ovariectomized females.

At 10 months.—Four mice represented this age group. Small cortical carcinomas were present in 2 animals bilaterally and in 2 unilaterally.

At 11 months.—A small cortical tumor was found in one adrenal of mouse 2266, which was killed at this age.

At 12 months.—Four animals were killed at this age. Adrenal tumors occurred in 3 of them unilaterally and in one bilaterally. All the glands containing neoplastic areas were considerably enlarged. Up to now the tumors had been composed of the large polygonal cells, but from this time on small cuboidal cells with deeply staining oval nuclei were present in many of the growths. As in the ovariectomized females, this second cell type appeared first between and beneath the fibers of the capsule of those adrenals in which tumors of the polygonal cell type were already present.

Older age groups.—In most of the neoplasms of the older age groups the large polygonal cell predominated and, judging from the number of mitotic figures, grew more rapidly. The small cuboidal cells showed a tendency to form rows and cords and to surround areas composed of giant cells with yellow pigmented cytoplasm. These “yellow giant cells” were consistently observed in the adrenals of castrated males, but did not seem to have any active part in tumor formation. Although they were present in the adrenals of gonadectomized females they were more numerous in the adrenals of the males.

In one adrenal of mouse 2529 (23 months) a small tumor was composed exclusively of cuboidal cells and yellow giant cells. In the opposite adrenal a large growth, composed mostly of large polygonal cells, was present; an area composed of nuclei scattered throughout a syncytium and similar to that shown in Fig. 6, was also present in this tumor.

Lung metastases were not noted in any of the castrated males.

In general the adrenal tumors of the gonadectomized females and males were fairly similar in structure. Points of difference were: (a) the cuboidal tumor cells first appeared at a later age in the males, 9 months in females, 12 months in males; (b) there were more “yellow giant cells” present in the tumors of the males; (c) judging from the size of the tumors those in the males grew more slowly.

The total percentage of adrenal tumors was 72.54 in the males and 91.90 in the females. There were 54.05 per cent of unilateral tumors in the males, and 31.03 per cent in the females. Bilateral tumors occurred in 45.94 per cent of the males, and 68.96 per cent of the females. Only animals more than 3 months old are included in these figures. Tables I and II give the results in tabulated form.

DISCUSSION 1

Carcinomas of the adrenal cortex are rare, and their occurrence in so many of the gonadectomized animals of the extreme dilution strain ce makes logical the conclusion that early removal of the gonads was responsible for their appearance. As it is recognized that some functional relationship exists between the adrenal cortex and the gonads, it is possible that the initial growth leading finally to neoplasia was an effort on the part of the cortex to compensate for the absent gonads. Whatever the initial reason, it is evidently characteristic of the extreme dilution strain ce, but not of other strains tested, dba and C57 black (4), where gonadectomy did not lead consistently to the occurrence of cortical carcinomas. There are 2 unique factors in the ce strain that might have had an important bearing on the occurrence of cortical tumors: the peculiar anatomical position of the adrenals, and a constitutional hypogonitalism.

It has been mentioned previously that the adrenals of the ce strain are in direct proximity to the kidneys, while in the dba and C57 black strains they are about 2 mm. farther cephalad. In embryonic development the adrenals originate from the celom ic mesoderm in the region of the genital ridge; in the ce mice they evidently remain closer to their original position, perhaps as an outcome of retarded development, for which histological proof was noted. At 1 month of age the cortex was not yet completely separated from the medulla, but penetrated it partially with finger-like projections.

1 The hormonal effects of the adrenal cortical tumors will be discussed in a separate paper by Dr. George W. Woolley.
As evidence for the second factor, namely, the constitutional hypogenitalism, the following observations are advanced: Data collected on the breeding behavior of ce mice showed that the females seldom had their first litters before the age of 4 or 5 months, that they stopped breeding earlier than females of some other strains, and that complete sterility frequently existed in both sexes. Histological examination of ovaries often showed a scarcity of ova. An

\[\text{Table I}\]

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The early changes and small tumors in the adrenals of the gonadectomized ce mice were found immediately beneath and in some cases between the fibers of the capsule. It is conceivable that the adrenals, which were retarded in development, contained some early components from the region of the genital ridge, and that these cells might have been kept undifferentiated and inhibited by sex hormones produced by functioning gonads. Perhaps they themselves were potentially capable of hormone production, and after the removal of the gonads attempted to compensate for them. Gonadectomy freed these undifferentiated cortical cells from inhibition and resulted in their uncontrolled proliferation. Likewise the ovaries may have contained similar cells from the genital ridge, which were inhibited when normal functioning ovaries were present. By this hypothesis a deficiency of sex hormone due to ovarian hypofunction may have resulted here, too, in an uncontrolled proliferation of the same type of cell.
If the above hypotheses are correct the proper sex hormone treatment might prevent the development of adrenal cortical tumors, or arrest them if they are already present. Further experiments should be carried out to test this assumption.

SUMMARY

Gonadectomy was performed at 2 days of age on 70 females and 61 males of the extreme dilution ce strain of mice. Adrenal cortical carcinomas arose in many of them. The total percentage of adrenal tumors was 91.90 in the females and 72.54 in the males. There were 31.03 per cent of unilateral adrenal tumors in the females and 54.05 per cent in the males. Bilateral adrenal tumors occurred in 68.96 per cent of the females, and 45.94 per cent of the males. Only animals more than 3 months old are included in these figures.

REFERENCES

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