A NEW TYPE OF ADRENAL TUMOR AND ITS COMPARATIVE HISTOPATHOLOGY

III. Serial Homologous Transplantation

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ABSTRACT

An undifferentiated adrenal tumor from a DMB-treated C₃H (Jax) female mouse was found transplantable in about 50% male and 75% female homologous animals used. The “takes” were better and faster in the females; the sex in which the tumor had originated. The latent period shortened with successive passages and was shorter for females. Histologically, the transplanted tumors resembled the original tumor but tended to be more pleomorphic, highly vascular, and showed increased mitotic activity. Evidence was found to show that testosterone which inhibits induction of adrenal tumors cannot prevent their proliferation.

INTRODUCTION

The induction and progressive development of this hitherto unclassified adrenal tumor have been described under Part II. The transplantation study was carried out for three reasons: Firstly, to find if the tumor was transplantable and to help establish its histogenesis. Secondly, to find if large subcutaneous growths would become secretory and affect the host animal by “corticoid” production. Thirdly, to study the growth pattern and other characteristics of the tumor kept under continuous transplantation over several passages; and to maintain the tumor in vivo.

MATERIALS AND METHODS

One of the large bilateral adrenal tumors from a 7:12 DMB-treated C₃H (Jax) female mouse described under Part II (Mody, Ibid.) was used. The tumor was transplanted subcutaneously by a fine trochar-needle in 10–12 weeks old homologous animals of both sexes. In all, 67 mice were used for transplantation through 6 successive passages (Chart 1).
The animals were palpated once a week near the site of transplantation so as to mark the beginning of growth. Usually, the growth could be felt with the finger tips when it was of pinhead size. This was considered as the time of tumor "take" for counting the latent period. The animals were killed when they looked emaciated due to the large tumors or because tumors frequently ulcerated through the skin.

**Chart 1.** Data on latent period, tumor "takes" and survival of the 67 C₃H (Jax) host mice used for transplantation. The blackened or crossed portion of each bar indicates the average latent period, while the total length of each bar denotes the average survival. The lower bars refer to the survival of mice in which the tumors did not take.

**RESULTS**

The data on tumor 'takes', the latent period and the survival are shown in Chart 1. The grafts were successful in 21/41 male and in 19/26 female B₄
mice and failed to 'take' in 20 males and 7 females. In both sexes the average latent period decreased with successive passages and was shorter in the female animals (Chart 1). It was 16 weeks in case of males and 13 weeks for the females. The survival and the latent period shorten with successive passages in both sexes.

The Transplanted Tumors

The tumors grew subcutaneously at the site of transplantation and varied from 0·5-3·0 cm. in their largest diameter in the different individuals. At autopsy, the tumor was found encapsulated and well supplied with large blood vessels. The growth was lobulated and whitish to pink in colour. The cut surface was smooth and the tissue was soft but firm.

The transplanted tumors resembled the original tumor (Figs. 9, 10 in Part II) in the essential histological structure. All the tumors were undifferentiated and the cell type was similar (Fig. 1) though pleomorphic. The cells in compact strands appeared as thin spindle nuclei which were darkly staining. In other areas they may appear mainly as sheets and whorls consisting of plump spindle or ovoid nuclei with well-dispersed chromatin granules (Fig. 2). In yet other areas the spindle cells had become highly pleomorphic or irregular. The nuclei appeared irregular, oval and empty looking (Fig. 3) or the nuclear details were not distinct. The cytoplasm was scanty and the cell borders were indistinct. The mitotic activity of the tumor cells was considerable (Fig. 3). Tiny capillaries were frequently seen in the tumor mass but in some areas the capillaries were highly dilated and papillae of the tumor tissue were seen perivascularly (Fig. 4). Such areas resembled a pericytoma in pattern. The tumors were rich in reticulum as was the original tumor. Occasionally, fine peri-cellular reticulum was also observed. Patches of myxomatous degeneration, hemorrhage and necrosis were found in several tumors.

The histological variations were mainly due to the pleomorphic nature and mitotic activity of the tumor cell growth. These quantitative differences in the successive passages and in the two sexes are shown in Chart 2. It can be seen that the transplanted tumors grew more pleomorphic with subsequent passages. After transplantation the mitotic activity became considerable in the females and by fifth passage had increased in the males also. In some of the tumors the cellular pleomorphy characteristic of these tumors was remarkable. There were histologically different neighbouring portions in a part of the tumor (Figs. 5, 6). These abrupt transitions were distinct. Even the reticulum distribution was different in the two areas.
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Chart 2. Quantitative differences in the pleomorphism of tumor cells at different passages. The differences marked on the bar denote the number of male or female mice used at each passage.
It was abundant where the cells consisted of spindle-shaped nuclei in sheets and much less where the cells consisted of ovoid or irregular nuclei in small groups and clusters.

The tumors were not metastatic. A few of the transplanted tumors which were studied for their "corticoid" content were found positive (Fig. 7) as were the original tumors (see Part II). Corticoids were also noted in the host adrenals (Fig. 8). However, the host adrenals and other organs did not show any histological change. Many of the animals showed non-specific changes in the livers. There was fine fatty infiltration, focal or diffuse degeneration and even patchy necrosis. Often the blood vessels were dilated and congested.

**DISCUSSION**

Dunham and Stewart (1953) in their survey of transplantable tumors refer to the transplantable adrenal cortical carcinomas of the well-differentiated type. These originated in the gonadectomised Ce strain mice (Woolley, 1946) and were estrogen or androgen secreting. Apart from this there is only one prior reference in literature, which is a paper by Dalton *et al.* (1943) on transplantation of a small spontaneous cortical tumor from an albino C strain female mouse aged 24 months. The tumor cells are described as possessing a nucleolus and seem to differ from the predominant spindle type nuclei of the undifferentiated tumors in DMB-treated mice described here.

The present study by serial homologous transplantation of this unusual type of adrenal tumor obtained from a DMB-treated mouse has yielded much information. It was discovered that the "takes" were better when a tumor was transplanted in the sex of its origin. It is not known whether this is true of other endocrine tumors as well, but the observation warrants further study to elucidate the reasons. It was noted that the proportion of male or female animals that "take" tumor at each passage was not correlated with the number of that passage. However, successive transplantations are being continued.

The latent period decreased with successive passages in both sexes. Again the latent period was shorter in the females so that the "takes" were not only better but faster in the sex of origin of the tumor. It has been found that the induction of these tumors in the adrenals was adversely affected by androgens (see Part II) which may explain the lesser number of successful "takes" and somewhat longer "latent period" in male animals. It is intended to carry out transplantations in castrated males.
to determine the differences. The survival and the latent period were co-related and appeared associated in direct proportion. This might be expected since if the tumors come up early the animal's survival would be shortened. The reasons why many of the female animals also failed to "take" the tumor in spite of good survival are not clear.

It was interesting to note that the essential structure of the original tumor was maintained in all the passages and in both sexes. The cell type was also similar but there were quantitative differences in the pleomorphism of cells. While the original tumor was mainly composed of spindle and oval cells the transplanted tumors showed a much greater pleomorphism of the component cells. More remarkable was the increased mitotic activity of the transplanted tumor cells; especially in the case of female animals. Thirdly, at times the transplanted tumors were very vascular and the capillaries were enormously dilated, congested and showed a number of polymorphic leukocytes. All the three differences stated above as departures from the original tumor might be in all probability due to transplantation per se, which allowed the tumor to expand freely in the loose subcutis and grow rapidly with consequent changes in morphology of fast-growing cell populations, ample vascularisation and eventually ulceration through the skin with hemorrhages and necrosis in parts.

Neither the original nor the transplanted tumors produced metastases. Applying special staining technique, "corticoids" had been detected in frozen sections of the original as well as the transplanted tumors (unpublished) but there was no evidence of "secretion". In fact no specific effects on any of the host organs were visible after several months of carrying large transplanted tumors.

The serial transplantation study has elucidated an important aspect of mechanism of carcinogenesis in these tumors. Earlier it was seen that the androgens inhibited induction of adrenal tumors and yet it was possible to grow these tumors in intact male mice. This shows that androgens prevent "initiation" of the tumors. Their mechanism of action is to inhibit the "A" cells from responding to the carcinogenic stimulus. The androgens have no influence on the proliferation of cells that have once become tumorous and hence these tumors can grow when transplanted in intact male mice.

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REFERENCES


EXPLANATION OF PLATES

PLATE XVII

Fig. 1. Transplanted adrenal tumor in the subcutis. The structure is undifferentiated as in the original tumor. C3H (Jax) female host. Age 10 months. H & E, × 150.

Fig. 2. Tumor cells in strands and whorls. The nuclei are darkly staining spindle type or ovoid with well-dispersed chromatin granules. Same tumor as in Fig. 1. H & E, × 600.

Fig. 3. Another area of the same tumor, showing pleomorphic cells. Irregular, oval, empty looking nuclei. Mitotic figures are seen. H & E, × 600.

Fig. 4. A different area in the same tumor, where the vascularity is great. The tumor cells are seen perivascularly. H & E, × 600.

PLATE XVIII

Fig. 5. An area of abrupt transition in the transplanted tumor. The cells on the right are plump, ovoid or spindle-shaped. The cells on the left are more pleomorphic and are in small groups and clusters. C3H (Jax) male host. Age 15 months. H & E, × 150.

Fig. 6. Another transplanted tumor showing abrupt transition in histological structure due to excessive pleomorphy of cells. C3H (Jax) female host. Age 12 months. H & E, × 150.

Fig. 7. "Corticoid" granules seen in the tumor cells of a stained frozen section. C3H (Jax) male host. Age 6 months, × 150.

Fig. 8. Adrenal of the above host also shows "corticoid" deposits, × 150.