THE CYTOLOGY OF FUNCTIONING ADRENAL CORTEX TUMORS

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The clinical manifestations of the endocrine activity of adrenal cortex tumors (hyperplasia, adenoma, carcinoma) have been outlined by Linser (31), Bullock and Sequeira (7), Guthrie and Emery (26), Apert (1), Gallais (17), Glynn (18), Van Dam (39), Berner (3), and Grollman (24). Some of the congenital tumors, especially the adrenal hyperplasias, liberate a growth hormone. A comprehensive review of cases of macrosomia interrenalis congenita, which is often combined with clinical manifestations in the sexual sphere, has been published recently by Bayer and Lang (2). The majority of the functioning tumors of the adrenal cortex influence the behavior of the external sex organs and the secondary sex characteristics. In this respect adrenal tumors may be divided into three groups: (I) virilizing, (II) feminizing, (III) indifferent.

I. Among the virilizing tumors three subgroups are recognized: (a) congenital, (b) juvenile, and (c) adult. In the congenital group the hormone virilizes the female fetus and causes pseudo-hermaphroditism, as in a case of adenoma of aberrant cortical tissue recorded by Marchand (32), and one of adrenal hyperplasia reported by Fibiger (15). Juvenile tumors are often anaplastic. They are more frequent in girls than in boys (Grollman) and lead to development of male sex characteristics, hypertrichiasis, acne, deepening of the voice, and enlargement of the external genitalia, often accompanied by a mild degree of obesity (obesitas praecox, Guthrie). In the male they may produce an increased muscular development (Hercules type, Guthrie) but leave the gonads unchanged. The adult tumors are known only in females and cause virilism and amenorrhea. Kepler's striking illustrations have been reproduced in the text-books of Grollman (24) and Cameron (9). In old women the virilizing effect may concern only the voice (R. Meyer, 33).

II. Contrary to the statement of Grollman, the existence of feminizing adrenal cortex tumors in the adult male is well established. Levy Simpson and Joll (36) give a survey of 5 unquestionable cases and a full report of a sixth remarkable case. In these cases the permanent or temporary improvement or disappearance of symptoms following surgical removal proves that the tumors were responsible for the feminizing effect.

III. Among the indifferent cortico-adrenal tumors a distinction must be made between adenomas and the carcinomas. Most of the typically benign cortical adenomas have no influence upon the secondary sex characteristics. Some large bilateral tumors are said to have caused destruction of the medulla with an associated arterial hypotension (Grynfeltt and Rimbaud, 25). It is of interest, in this connection, that the virilizing tumors are nearly always associated with hypertension (Gordon and Browder, 22) and a mild degree of arteriolar nephrosclerosis (Boyd, 5; personal observation). A list of the in-
different adrenal cortex carcinomas appearing in the literature is given in Van Dam's (39) excellent paper, but his experience as well as ours suggests that caution is needed in establishing this diagnosis. Bilateral tumors are often metastatic. When there are extensive mediastinal and lung metastases it may be difficult to determine the primary seat of the neoplasm. As Van Dam points out, in these doubtful cases the histologic features are of little help. Further studies of these indifferent tumors are needed.

As to the biological properties of virilizing tumors it must be emphasized that the symptoms are not caused by an excess secretion of cortin. This question has been discussed in an earlier paper (21). Levy Simpson (36), Levy Simpson, de Fremery and Macbeth (35), Slot (37), Kepler (29), and Hare (27) found an excess of comb-growth hormone in the urine of patients suffering from adrenal virilism, and Broster and Vines (6) isolated a new compound \((C_{23}H_{37}O_3)\) from the urine of such patients. An estrogenic hormone is also present, but only exceptionally in excess. Frank (16), Cahill (8), and Slot (37) found no excess of comb-growth hormone in the virilizing tumor itself, but this does not, of course, exclude the possibility of the tumor giving rise to the hormone, since no storage process is observed in the stimulated adrenal cortex. Removal of the tumor, if it is the source of the hormone, should be followed by a drop in the urinary excretion of the latter. We have found no case recorded of a virilizing tumor in which this occurred, but convincing evidence of such a drop has been given by Levy Simpson and Joll (36) after the removal of an adrenal feminizing tumor. In their case the excess of estrogenic hormone disappeared after the removal of the carcinoma but returned with the development of metastases and a recurrence of the symptoms. This supports the theory that the hormones directly responsible for either masculinization or feminization are formed in the tumor itself. There is little to be said in favor of the opinion that the adreno-genital syndrome is brought about through the mediation of the gonads. From these biological investigations it must also be concluded that virilizing and feminizing tumors of the adrenal cortex secrete different hormones.

Are there essential differences in the cytological characteristics of the three types of adrenal cortex tumor? The purpose of this paper is to examine this question. With a few exceptions, to which we refer later, the histologic reports we have found in the literature lack precision and are inadequately illustrated. We have thus been obliged to concentrate on the study of our own material, which is necessarily limited. Owing to the rarity of the feminizing tumors we have made use of the very thorough description of Levy Simpson and Joll's case (36). In view of these circumstances we have had to be guarded in our conclusions, but we hope that our observations will stimulate further research.

VIRILIZING TUMORS

CASE 1. *Virilizing Adrenal Tumor in a Girl of Twenty-one:* Material for this tumor was sent to me by W. M. Dickson, who had operated in the case for Dr. Levy Simpson. By the courtesy of Dr. Hare, who was in charge of the pathological examination, I am able to publish the result of my own investigations.

The bulk of the tumor has a trabecular structure which resembles closely the zona reticularis of the human adrenal cortex. The trabeculae are narrow and surrounded by an intricate network of capillaries; beneath the capsule of the tumor spongiocytic cords are present.
In the specimens examined by me there were no definite evidences of atypical growth. A few multinucleated cells of moderate size were present. Mitotic figures were rare.

We shall not attempt to say here whether this tumor was a benign or a malignant adenoma, but shall devote our attention to the cytology. The cytoplasm was strikingly heterogeneous, especially when stained with iron hematoxylin or with Masson's trichrome technic (Fig. 2). The siderophil aspect of the cytoplasm is due to the accumulation of mitochondria (Fig. 2, a, c, d). These coalesce to form darkly stained granules which accumulate at one pole of the cell around a conspicuous centrosome. Gradually a colorless halo surrounds these granules, and an irregular vacuole is formed, in which the granule loses its basophil staining properties (Fig. 2, c) and finally fades away. At this stage a fringe of large vacuoles can be seen at one pole of the cell. There is evidence that the contents of these vacuoles are eliminated in the lymph spaces (Fig. 2, c). In other words, the cytology of this tumor favors an endocrine activity.

Case 2 (No. 1437). Virilizing Tumor of the Right Adrenal Cortex in a Boy of Six, with Metastatic Nodules in Liver and Lungs (21): The chief symptoms in this patient were moderate obesity, the presence of a beard and mustache and of pubic hair, acne, and striae. The penis and testicles were of normal size. The right adrenal was of the infantile type. The tumor was unquestionably an anaplastic carcinoma, devoid of a trabecular structure in most places. The cells and nuclei varied considerably in size, and numerous giant cells were present. The connective stroma was scanty in wide areas. Where it was abundant the tumor looked like an alveolar carcinoma with large polygonal cells. Similar areas were found in one of the indifferent adrenal carcinomas to which we refer later. Necrosis with calcium deposits was marked in the primary tumor. In the small, well vascularized metastatic nodules were limited areas with a trabecular structure and different cell types, which
FIG. 2. CASE 1: VIRILIZING ADENOMA IN A GIRL OF TWENTY-ONE

a. Accumulation of mitochondria at the vascular pole; vacuoles at the opposite pole.  
b. Marginal vacuoles containing siderophil granules.  
c. In one of the cells, coalescence of mitochondria; in the other large vacuoles containing granules partly siderophil, partly fuchsinophil. These two cells are in contact respectively with a capillary and a lymphatic.  
d. Cell implanted on a capillary; mitochondrial accumulation at the vascular pole; granules and vacuoles at the opposite pole.

FIG. 3. CASE 2: VIRILIZING CARCINOMA IN A BOY OF SIX YEARS

a. Marginal vacuoles and siderophil granules.  
b. Marginal vacuolation around a centrosome.  
c. Group of three cells, two of which show siderophil granules and vacuoles. Note connections with capillaries.
may be classified as follows: (a) cells with compact homogeneous cytoplasm; (b) regular vacuolated cells resembling the spongiocyte; (c) cells with siderophil or fuchsinophil granules (Zenker-Formol fixation) included in irregular vacuoles and arranged at one pole of the cell around a centrosome (Fig. 3). The latter type of cell resembles closely the cell which predominates in Case 1 (Fig. 2). Cells of this type were grouped in trabecular and well vascularized islets forming endocrine units readily distinguishable from the surrounding more anaplastic areas. The siderophil granules accumulated in the cell opposite the vascular pole; faintly stained granules were found between the cells.

FEMINIZING TUMORS

We have never encountered a feminizing adrenal tumor, but have had the opportunity of examining microscopic sections of Levy Simpson and Joll's case, in Miss L. M. Hawksley's laboratory in London (Cancer Hospital). The main features of her report (36) are reproduced here.

FIG. 4. LUTEAL CELL OF PREGNANT BITCH FOR COMPARISON WITH FIGS. 1, 2 AND 3

CASE 3. Feminizing Adrenal Cortex Tumor in a Man Aged Thirty-four: "In some parts there is a well-marked resemblance to a normal adrenal cortical structure, with differentiation in all three zones, while through the bulk of the tissue a structure approximating to one or other of the zones is readily recognized. Endothelium-lined blood channels separate the smaller groups and wider columns of tumor cells; the latter, when cut transversely, having an apparently perithelial arrangement. Variations in the staining qualities include a finely granular acidophilic cytoplasm which predominates in the well-vascularized tissue, and a high degree of vacuolation in the larger strands and plaques. No fuchsinophil cells have been detected. Scattered intra- and extracellular granules of golden-yellow pigment are present in variable amounts." Miss Ross, who has also examined this tumor, confirms the absence of siderophil and fuchsinophil granules.

INDIFFERENT TUMORS

In this group the adenomas must be separated from the carcinomas. Adenomas of moderate size are frequently observed, most of which do not interfere with the sexual characteristics.

CASE 4 (No. 1665). Adrenal Cortex Adenoma (pigeon egg size) of Right Adrenal in a Man, Forty-nine Years Old: This patient had a voluminous papilloma of the larynx, with asphyxia and cardiac hypertrophy and post-mortem evidence of chronic cardiac failure and mild arteriolar nephrosclerosis.

The adrenal tumor was composed of cell cords radiating from the center. The cytoplasm of the central cells was homogeneous and contained mitochondria. In the outer zone the cells showed a graded accumulation of small regular vacuoles until a spongiocyte type of cell was formed. The glomerular layer was present under the connective-tissue capsule.
We looked carefully for siderophil and fuchsinophil granules in the central area of this and several other adenomas. Occasionally a discrete coalescence of mitochondria was observed, but numerically the importance of the process leading to the formation of siderophile granules is negligible. In other words, the sexually indifferent cortical adenomas reproduce the structure of the normal cortex with the exception of the juxtamedullary zone, which we have described in an earlier paper and which contains the granules referred to (19).

We have in our collection two tumors from male patients labelled as cortico-adrenal carcinomas, which exerted no influence on the sexual organs. A careful examination of our protocols obliges us to consider the diagnosis in these cases with caution, as is true of almost every indifferent adrenal cortex tumor mentioned in the literature, Winkler's (41) cases included.

**Case 5 (No. 2609). Tumor of the Left Adrenal Weighing 90 Grams in a Man of Sixty-nine:** This patient showed metastatic involvement of the retroperitoneal, mesenteric, and mediastinal lymph nodes and of one of the left inguinal nodes. There was a nodule the size of a hazelnut in the right lung hilus in contact with the right bronchus, and another implanted on the tentorium cerebelli. From this enumeration it must be admitted that a diagnosis of a primary lung tumor cannot entirely be dismissed. The brain metastasis especially favors this interpretation.

The nodule in the lung hilus, however, is small and its yellow color and festooned aspect on section are rather uncommon for a tumor of bronchial origin; nor does it show any signs of aggressive growth towards the lung parenchyma. In appearance it is definitely metastatic. In almost every case of mediastino-pulmonary tumor which we have examined, including the oat-cell tumors, a process of metaplasia was present. This is lacking in the neoplasm under consideration. It is a very cellular growth with a scanty stroma. The comparison between frozen and paraffin sections is instructive. In the former the cords are narrow and compact; in the latter they are often disintegrated so as to resemble in some respects a "retrothel-sarcoma." The process of imbedding in paraffin has loosened the structure of the cords, a feature regularly observed in human adrenals post mortem. At a high magnification minute granules stained by the Smith-Dietrich technic are occasionally found in the cells. The stroma on the other hand is rich in lipoids (Smith-Dietrich). In areas where the cords are narrow and unaltered by fixation and imbedding, the analogy with the glomerular zone of the adrenal cortex is rather convincing. This impression is supported by the dust-like appearance of the nuclear chromatin. The cytological features of the tumor cells can be summarized as follows: cytoplasm scarce; vacuolation vague and exceptional; siderophil and fuchsinophil granules absent.
CASE 6 (No. 2169).  Malignant Carcinoma of Both Adrenals in a Man of Fifty-three Years, in Cachectic Condition: In this case there were no endocrine disturbances in the sexual sphere. The adrenals were the size of an adult fist. The lymph nodes of the celiac region were involved. Numerous metastatic nodules were present: one the size of a hazelnut, in the left lung hilus; one in the base of the left lung, attached to the diaphragm; several in the mediastinum and in the left deep lymph node chain of the neck. There was also right renal dystopia.

Here again there is room for several interpretations as to diagnosis. Histologically the tumor is an alveolar carcinoma composed of large polyhedral cells with a fine granular acidophil protoplasm and fairly rich in collagen. The vascular stroma is well developed. The histologic evidence is in favor of an adrenal tumor of cortical origin. There is a distinct similarity between it and the non-endocrine areas of the tumor of Case 2. Moreover, spongocytes are occasionally found. This tumor is devoid of any endocrine features. No siderophil or fuchsinoophil granules were found (Fig. 5, b).

To summarize, in our limited series the virilizing, feminizing, and indifferent adrenal cortex tumors differ definitely from one another. Even when metaplastic, the virilizing tumors contain endocrine islets with a well developed capillary network. Lymph spaces are conspicuous. In the cells of these islets the lipoid material undergoes complex changes leading to the formation of siderophil and fuchsinoophil granules which accumulate at one pole of the cell around a centrosome. In the feminizing tumor of Levy Simpson and Joll these granules are absent, while yellow pigment granules are present. Two indifferent tumors, probably of cortico-adrenal origin, are devoid of endocrine features and contain no siderophil, fuchsinoophil or fatty pigment granules.

DISCUSSION

In order to set forth clearly the significance of these observations, it is necessary to refer to one of our earlier papers on the structure and reactions of the human adrenal cortex (19). In this paper, which has had only a small circulation, but the conclusions of which are referred to in Cowdry's (12) and Bouin's (4) text-books, we sought to prove that the human adrenal cortex is composed of two zones: (a) a juxtamedullary zone, large at birth, rather inconspicuous in male adults, and reacting in females to pregnancy, the menopause, and old age; (b) the remainder of the cortex, concerned with the storage of cholesterin esters and undergoing complex changes in septic and toxic conditions (10, 11). The work of Howard-Miller (34) and of Ruth Deanesly (13) on the adrenal cortex in the mouse is also in favor of the functional individuality of the juxtamedullary zone. According to Deanesly, at three weeks old the adrenals are alike in the two sexes. At this time an inner dark staining cortical zone—called the x zone—can be distinguished. Growth of this zone has ceased in the male by the age of five weeks, but in the female it continues to grow until puberty. Later it degenerates slowly in the unmated animal. A new inner zone may arise later in the cortex; it also is of a transitional character. Castration causes the growth of an inner x zone in the male. In a recent book Grollman (24) has emphasized the individuality of the juxtamedullary zone by naming it the androgenic zone. We believe at present that this zone is quiescent in the normal adult male. Peripheral vacuolation is discrete. It is this zone which in man contains, besides pigment, the siderophil and fuchsinoophil granules characteristically arranged in the cells (19). According to
Ross (36), who examined a small series of adrenals, the granules are present in males during and after puberty (confirmation of our view, 1922), but do not appear in the female until after the menopause.

In 1922 we pointed to the probability of the presence of these granules in virilizing adrenal tumors, of which we had no personal experience at the time. Broster and Vines (6) have called attention to the fuchsinophil staining reaction of the juxtamedullary zone in the adreno-genital syndrome. Miss Ross (36) has examined with great care the granules found in the virilizing tumor of Hare's case. "With Bensley-Cowdry's technique," she says, "they were fuchsinophil; with Haidenhain's haematoxylin they stained black. These granules were found not only in the anaplastic areas of the primary tumor but also in the secondary growth obtained post-mortem. Helly-Maximow's fixative was used. In Lescher's case these granules were also present."

It is evident that in the cases of virilism examined by us the tumor cells tended to develop into a specialized type closely analogous to the cells of the juxtamedullary androgenic zone (compare Figs. 1, 2, and 3). This is not the case in indifferent cortico-adrenal tumors (Fig. 5 a, b). With regard to the presence of yellow, probably fatty pigment in the feminizing tumor of Levy Simpson and Joll, we doubt if it is of any significance. In our paper of 1922 we did not devote much attention to the distinction between pigment and siderophil granules. This point deserves further investigation.

While emphasizing the resemblance between the cytology of virilizing tumors and the androgenic zone, we must not neglect another analogy. Long ago Mulon (quoted by Goormaghtigh, 20) showed that the siderophil bodies (Guyiesse) found in the inner layer of the cortex of the guinea-pig are also present in the corpus luteum of that animal. The cytology of Case 1 is strikingly similar to that of the luteal cell of the dog in an early stage of pregnancy (Fig. 4) and of the human corpus luteum of pregnancy. New light is thrown on this analogy by the recent work of R. T. Hill (28) and of Deanesly (14), who have proved that the ovary secretes a male hormone. Deanesly has suggested that there may be a relation between thecal luteinization and the masculinizing effects of cooled ovarian grafts in the ear. Moreover progesterone is known to be chemically similar to other androgenic compounds and Greene, Burill and Ivy (23) claim that in large amounts it is androgenic.

From the cytologic point of view the juxtamedullary cells of the adrenal and the luteal cells of the ovaries have something in common (compare Figs. 1 and 4). Both transform the lipoid materials (among which are the cholesterol esters) into a colorless substance which is accumulated at the periphery of the cell as ill defined vacuoles, and seems to be eliminated in the lymph spaces. In the process of this lipoid transformation siderophil and fuchsinophil granules are formed. Since these granules have been found in all the adrenal virilizing tumors where a search has been made for them, we suggest—with the caution imposed on us by the small number of observations available—that these cytologic features express the androgenic activity of the cortico-adrenal and luteal cell. With this provisory conclusion in mind it would be interesting to compare the adrenal cortical tumors to the virilizing ovarian tumors described by Tuffier and by Robert Meyer (33). Masson, who examined the tumor of Tuffier's case (38), found that its cells had a striking resemblance to luteal...
cells and also, to a certain extent, to cells of the adrenal cortex. Some of the andreioblastomas of R. Meyer's series showed areas composed of interstitial cells which have probably cytological features in common with the cells of the virilizing adrenal tumors under discussion.

While we are aware of the fact that no definite conclusion can be drawn from the study of such a limited number of cases, we believe that further study of functioning adrenal tumors may bring forward a valuable contribution to the problems of endocrinology.

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