Androgen-Estrogen-induced Tumors

I. The Flank Organ (Scent Gland) Chaetepithelioma of the Syrian Hamster*

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SUMMARY

The morphogenesis and hormonal relationships of a unique malignant basal-cell epithelioma in flank organs of the Syrian hamster are described. The life history of this androgen-estrogen-induced tumor involves three stages: 1. The migration of hair matrix cells into dermal sheaths and/or dermal papillae to form epithelial nodules which become surrounded by connective tissue lamellae; these lamellated corpuscles occur normally in males aged 185 or more days, but not in females; they require the presence of androgen and are inhibited by estrogen. 2. Under the influence of exogenous androgen they increase in size, up to approximately 1 mm. in diameter, and the central cores of epithelial cells migrate peripherally as radiating cords. Under the influence of exogenous androgen and estrogen together, the stage 2 nodules increase in size and fuse to form definitive stage 3 neoplasms, the mean latent period for induction of which is ca. 230 days, with a range of 110–500 days. Once formed neither the spontaneous nor the induced tumors ever undergo complete regression, even with estrogen treatment. With androgen treatment alone stage 1 nodules occur precociously, and induced nodules appear in females and in gonadectomized or hypophysectomized hamsters. The effects of surgical interference, and of other hormones, upon the induction and behavior of the tumor are described, as are its growth characteristics in both hormone-free and hormone-containing diffuse cell and organ cultures. The chaetepithelioma metastasizes and is transplantable as a hormone-dependent tumor. Specific hormone activity at the tissue level, rather than “hormonal imbalance,” is considered critical in the induction and growth of this tumor. During 21 serial passages the transplanted tumor has not become autonomous.

Some semantic problems in oncology and possible modes of hormone action in tumor induction and subsequent growth are discussed in relation to this tumor. The value of the neoplasm in studies of carcinogenesis is emphasized.

One of the difficulties encountered in morphological, experimental, and chemical studies of carcinogenesis concerns the difference between late hyperplasia and early neoplasia. It would be desirable to have for such studies an experimental tumor in which a clear-cut, preneoplastic structure could be seen to originate from some component or components of a pre-existing, normal tissue or organ, and then to progress more or less slowly and predictably through recognizable changes to a definitive malignancy. Such a tumor may be induced readily in the flank organs of the Syrian hamster (Figs. 1–3).

In adult golden hamsters these organs are usually spherical or ovoid, slightly raised, heavily pigmented scent glands, bilaterally situated in a costo-vertebral position (Fig. 1). Although present at birth, they do not attain full size until sexual maturity, when they measure about 8 × 7 mm. in diameters in males. They are androgen-sensitive and therefore larger and more conspicuous in mature males than in females. Histologically, they con-
We regard this tumor as a specific kind of adnexal or subepidermal, from one to five animals in a cage. Untreated, mature females were caged individually, since they are more aggressive than untreated males or treated females. All were given water and Purina Laboratory Chow or Wayne lab-blox ad libitum with greens added twice a week. Room temperatures were maintained at about 75°F.

The surgical procedures employed were essentially similar to those used on rats (21). Tumor tissue was prepared semi-quantitatively for transplantation by forcing it through a stainless steel screen of No. 013-gauge wire, 30 meshes to the inch, into a previously weighed vial containing 1 ml. of physiological saline solution, which was then reweighed and diluted to a concentration of 5 mg/ml, and injected in 1-ml aliquots per animal. Unless designated specifically, stilbestrol and hormones were implanted subcutaneously as pure 20-μg. compressed pellets (30-μg. for testosterone propionate). Since the mean daily absorptions were 0.11 mg. for stilbestrol and 0.15 mg. for testosterone propionate, new pellets were routinely implanted in all animals treated longer than 150 days to insure continuous absorption.

Tissues were fixed routinely in Bouin’s fluid, embedded in paraffin, sectioned at 7 μ, and stained with hematoxylin and eosin or by the Mallory azan procedure. A number of serially sectioned flank organs were stained by the Van Gieson technic. Sections up to 80 μ in thickness were employed for special purposes. For dopa oxidase (tyrosinase) the methods of Laidlaw (47) and of Becker, et al. (9) were employed. The periodic acid-Schiff reaction and alcin blue were used for mucopolysaccharides. Foot’s silver ammonium carbonate method was employed for reticular connective tissue fibers. For nerve fibers Bodian’s protargol, Holmes’ silver nitrate, and supravital methylene blue methods were used.

Various fixatives, other than Bouin’s fluid, were used for special purposes—e.g. Bouin with trichloroacetic acid, Helly, Zenker acetic, and Zenker stock, for histological detail; Champy and Kolatchew-Nassanow for cytological detail; formol-acetic-alcohol, picro-saline solution, and et al. (9) were employed. The periodic acid-Schiff reaction and alcin blue were used for mucopolysaccharides. Foot’s silver ammonium carbonate method was employed for reticular connective tissue fibers. For nerve fibers Bodian’s protargol, Holmes’ silver nitrate, and supravital methylene blue methods were used.

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The only species used in this study was the Syrian hamster, *Mesocricetus auratus*, including a partial albino variety (68, 69), in addition to the usual golden form. The animals were secured from several Californian dealers and from our breeding colony; none were from inbred stock. They were maintained in cages with wire bottoms, from one to five animals in a cage. Untreated, nature females were caged individually, since they are more aggressive than untreated males or treated females. All were given water and Purina Laboratory Chow or Wayne lab-blox ad libitum with greens added twice a week. Room temperatures were maintained at about 75°F.

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In looking for inconspicuous differences in some historical preparations a comparison eyepiece was useful.

Detailed tabularized data have been excluded from this paper but may be obtained from the authors upon request.

**MATERIALS AND METHODS**

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**OBSERVATIONS**

**General Introduction**

Grossly, a flank organ tumor is first evident as a minute, palpable nodule usually in the periphery of the organ. As a rule it is single, but multiple tumors occur. Nodules may appear at the same or different times in the two organs. The earliest palpable nodules are best seen following reflection of the skin (Fig. 5). As they enlarge they remain more or less spherical, and eventually extend well beyond the maximum diameter of the flank organ and attain...
weights of 5 or 6 gm. (Fig. 3), although a weight of over 11 gm. may be reached (Fig. 6). Ulcération through the covering epidermis may occur among the larger tumors (Figs. 7, 8).

The tumor has been traced through a series of developmental stages to an origin from the roots of flank organ hair follicles (Fig. 9). The definitive tumor arises from smaller nodules, which may become large enough to be seen from the dermal side of flank organs. Many such nodules occur in flank organs of hamsters treated with androgen, as well as with treatment with combined estrogen and androgen. With the latter treatment, however, further growth into definitive tumors continues; with androgen treatment alone, little or no further growth occurs. In animals treated for shorter periods (Table 1) these tumor nodules may be traced through one or more microscopic nodules to morphologically different, laminated structures, clustered mainly between the bulb and the insertion of the arrector pili muscles of hair follicles (Fig. 10). These structurally characteristic bodies constitute a distinct preneoplastic phase in the development of the tumor. Such laminated structures can be traced still further, through nonlaminated, compact cellular masses arising directly from hair matrices (Fig. 11).

On the basis of the above brief résumé the development of the flank organ tumor will be considered in three stages. Stage 1 refers to the laminated corpuscles (Fig. 12), which occur normally in untreated, intact males (Table 2), as
essential to the origin and development of the chaetepithelioma.

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Vol. 24, October 1964

well as in young animals of either sex treated with androgen, with or without estrogen (Table 1). They have a maximum diameter of about 100 µ. Stage 2 refers to a nonlaminated, microscopic or barely macroscopic, mass (Fig. 13) found only in animals treated with androgen alone, or in combination with estrogen. Stage 2 nodules vary in size, with diameters from ca. 100 to 500 µ. Stage 3 refers to the palpable neoplasm found only in androgen-estrogen-treated animals (Chart 1).

With this background, histological descriptions of the three stages in the continuous ontogeny of the flank organ tumor will be given below, and the hormonal factors involved in each stage will be presented.

Specific Considerations

Morphology and morphogenesis.—

Stage 1: A typical corpuscle consists of a central core or cluster of small, closely packed, more or less cuboidal cells enclosed by a conspicuous shell of several loosely arranged, more or less concentric laminae composed of collagenous connective tissue fibers (Fig. 12). No elastic or reticular fibers are present. The corpuscles are usually amelanotic; melanin may occur throughout or it may be confined to the central core or to some of the laminae. It appears to be in dendritic cells.

No matter how abundant or structurally varied the corpuscles may be, nothing comparable has been observed in other portions of the skin, even in animals subjected to heavy, prolonged treatment with sex hormones.

Between the 24th and 25th days of life, it is possible to detect a slight sexual dimorphism in flank organs. This difference gradually increases until it is clearly recognizable at the 32d day; by the 35th day it has become relatively conspicuous. The dimorphism consists of greater enlargement in males of sebaceous glands, hair bulbs, and dermal papillae. By the 32d day tiny clusters of cells may occasionally be seen forming caps at the apices of some of the hair bulbs of males (Figs. 14-16). These cell clusters become more prominent with time, and new ones continue to form. This condition is not observed in females unless they have been androgenized. The clusters lie in the regions where remnants of earlier papillary rests might be expected were such remnants to persist from an early growth phase—i.e., anagen I (13). Such caps continue to form in the same manner throughout the life of the animal. Favorable sections reveal continuity of caps with matrix. The most obvious examples reveal direct invasion by matrix cells through a more or less disorganized basement membrane into the region of the cap (Figs. 11, 17). Alternatively, some matrix cells and occasionally some melanin-containing cells appear to reach the cap via the papilla. In such cases it is impossible to distinguish, histologically, many of the cells in the enlarged papillae from those in the adjacent matrix.

As the follicles shorten during transition from the growing to the resting stage of the hair cycle (i.e., catagen [13], and during the ensuing resting, i.e., telogen phase), the caps tend to retain their positions relative to the hair bulbs. As the follicles elongate after telogen—i.e., during the next anagen part of the cycle—the caps become detached and lie in the connective tissue lateral to the bulb and later, with continued elongation of the follicle, lateral to the keratogenous zone (Fig. 10).2 Despite the elaboration of a connective tissue laminated shell no argyrophilia suggesting new fiber deposition has been observed in these young preneoplastic nodules.

During the separation of the cap from the bulb and the subsequent formation of laminae about it, relatively dense portions of the dermal sheath may remain around the central cell cluster tending to incompletely isolate the core from the laminae (Fig. 18).

As males increase in age many caps are formed and become detached from follicles, so that large clusters of stage 1 nodules may be observed adjacent to follicles of old animals (Fig. 10). In estrogen-androgen-treated animals similar clusters may be seen when stage 2 or even stage 3 tumors are present. Undoubtedly many of these nodules are of different ages, but it possible that some stage 1 corpuscles lie relatively dormant while others enlarge through stages 2 and 3 (Fig. 19).

In estrogen-androgen-treated animals it is not at all uncommon for matrix cells, migrating into dermal papillae, to fail to “escape” through the stalk. When this happens they form intrafollicular, cellular, stage 1 or even stage 2 nodules, which may become quite large (Figs. 20, 21). Such incarcerated nodules eventually become free, either by the retreat of the enclosing follicle during catagen or by follicular degeneration (Fig. 22). A “freed” stage 1 nodule may sometimes retain its connection with the parent follicle (Fig. 23), or its remnant, until after it has developed into a large well organized stage 2 (Figs. 24-28), or even stage 3, tumor. In such cases mitoses may continue in the distorted matrix, and it is probable, in certain instances (Fig. 26), that there is a continued matrix contribution to the growing tumor. Whether or not follicles are involved in such retentions, they eventually become very abnormal and frequently cystic (Figs. 22-24), cease to produce hairs, and are frequently filled with squamous-cell debris. The epithelium of these follicles is similar to surface epidermis, with the basal cells resembling those of the stratum germinativum rather than those of external root sheath or matrix.

1 The plucking of club hairs during the extremely short period when they occur (Fig. 4) would not be expected to increase tumor incidence, since exogenous androgen itself is followed by a burst of pilary growth (30).
Since the definitive tumor possesses some characteristics suggestive of a nonepithelial origin, histogenetic pathways alternative to the one described were investigated. Although it is possible to find early stages of the corpuscles in contact with myelinated nerves, the relationship is not significant. The use of the Bodian and Holmes' silver methods and of intravital methylene blue disclosed no evidence suggestive of an origin from elements of neural sheaths. Similarly, the use of the dopa oxidase technic and of tyrosine incubation indicated that the tumor was not an unusual type of melanoma. That the growth of the primary tumors is independent of local melanin is indicated by the fact that transplants and pulmonary metastases from them are always unpigmented even when derived from heavily pigmented primary tumors.

It is noteworthy that each normal flank organ, with a certain amount of surrounding skin, constitutes a "field," in the sense in which this word is used in experimental embryology. Under the influence of chronic androgen treatment flank organ diameters increase, the hair follicles and sebaceous glands of the skin surrounding the flank organs enlarge, and the pigmentation of the scent glands not only becomes denser but is extended peripherally. It is our impression that this enlargement of the flank organs at the expense of the surrounding skin is most rapid during early treatment, following a short initial lag period. During prolonged treatment the rate of this growth gradually decreases to an ultimate standstill. Following removal of exogenous androgen, regression of each flank organ to the normal level occurs, or in castrates to markedly subnormal levels.

Although androgen results in an increase in flank organ area at the apparent expense of surrounding skin, extirpation experiments demonstrated that the bordering skin is in itself incapable of forming new flank organ tissue or any of the tumor stages associated with androgen or androgen/estrogen treatment. On the other hand, autotransplantation to the head of even small fragments of scent gland, devoid of marginal skin, from 50-day-old, untreated, animals show no enlargement, and certainly no surrounding tissue pre-emption, during the life of the recipients, although hormone treatment results in the induction of microscopic tumors in these fragments.

Stage 2: When treatment with androgen is prolonged some stage 1 corpuscles fuse to form single masses (Fig. 27). As corpuscles enlarge or fuse, epithelial cells tend to become rearranged into radial cords separated by connective tissue (Fig. 28). This connective tissue derives principally from the peripheral shell, with possible contributions de novo. This radial arrangement of the cords is one of the most striking structural characteristics of later stages (Fig. 29), including metastases (Fig. 30).

All these nodules do not arise from fusion of stage 1 corpuscles. Some cells in the central cores migrate peripherally among the fibrous laminae to form small stage 2 nodules. These transitions between stages 1 and 2 may enter prolonged dormant phases at any time during their development. Like matrix cells and stage 1 nodules, stage 2 tumors contain no glycogen, but associated connective tissue fibers are periodic acid-Schiff-positive.

Stage 3: Following the addition of estrogen to the androgen, stage 2 masses enlarge. When estrogen and androgen are combined from the start, expansion and/or fusion of stage 2 nodules does not cease after a certain size has been attained but continues until single, lobulated, definitive tumors are formed. Larger tumors usually consist of elaborately crumpled or folded membranes, recognition of which may be difficult in some of the denser, more compact specimens. As stage 3 tumors enlarge, overlying hair follicles either disappear or become displaced peripherally.

These firm, somewhat elastic, lobulated stage 3 tumors may be shelled out readily (Fig. 31) from the surrounding connective tissue but must frequently be torn loose from a strong attachment to the overlying epidermis. They show very little tendency to infiltrate the surrounding tissues, although their capsules are delicate. Their sectioned surfaces are slightly viscid, somewhat translucent, and of a faintly bluish-white color. Microscopically the arrangement of epithelial nuclei may show conspicuous regimentation suggestive of type I neurilemmomas (Fig. 32). Arrangement of cells and fibers in whorls (Fig. 33) may be observed also.

Radial sections of individual lobules show radiating cords of cells. Centripetally, the collagenous fiber stroma about the cords is relatively dense, the cords narrow, and tumor cells sometimes are isolated in nests. Within nests the cells may show degenerative changes. Centrally the stromal strands become progressively more slender, frequently disappearing before reaching the outer capsule. The intervening cords of tumor cells become wider, fusing into a narrow, more or less continuous, serrated cellular subcapsular layer (Fig. 29).

In some larger tumors, in certain regions of small ones, or in metastases or transplants, the cords of cells may become more or less broken up by thickening of the supporting connective tissue stroma (Fig. 34) which retains its characteristic staining affinity for picric acid, aniline blue, and Schiff reagent. Some of the larger tumors may possess areas of fibrosis and hylainization, sometimes including cartilage or bone. Areas of extensive hemorrhage and/or necrosis are not characteristic.

When stage 2 nodules fuse to form larger tumors, intervening loose connective tissue with its various cellular components—e.g., fibroblasts, macrophages, mast cells, melanocytes, lymphocytes—becomes incorporated within the neoplasia; even muscle fibers from the panniculus carnosus may become enclosed within the laminated cortex of stage 1 nodules and presumably within the structure of later stages.

The early melanin content may increase in some tumors and disappear in others, so that large tumors vary from highly melanotic to amelanotic masses; as a general rule they consist of mixtures (Fig. 35). With one ambiguous exception no keratinization has been observed within the tumor. Even the yellow flavin dye, 9-phenyl-5,6-benzoiso-alloxazine, known to color keratinizing cells, failed to demonstrate keratin in the tumors.

Induction, incidence, and hormonal relationships.—Although the definitive tumor (stage 3) requires combined androgen and estrogen, earlier stages (1 and 2) require only androgen. Even physiological amounts of androgen...
are sufficient to induce stage 1 nodules with time—e.g., approximately 100 per cent of untreated males 185 days or more of age possess them. The amount of endogenous androgen in females appears to be inadequate in this respect, since even the oldest (e.g., 1000 days) of untreated females fails to show them. Stage 1 nodules can be increased in number, induced to occur at significantly earlier ages, and caused to metamorphose into stage 2 tumors by increasing the amount of androgen. Except in the presence of exogenous androgen, estrogen prevents tumor induction.

Increasing the amount of androgen tenfold (ten 20-mg. pellets per 150 days) over that customarily used for stage 2 development fails to cause further development. Only when exogenous estrogen is added to the system are stage 2 nodules promoted to definitive neoplasms. The presence of estrogen at an earlier period neither stimulates nor retards the development of stages 1 and/or 2 nodules. Approximately one-half of all androgen-estrogen-treated animals (males, females, and gonadectomized) show some stage of chaetepithelioma development after 75 days of treatment (Chart 2). At least one-half of such animals show stage 3 tumors after 230 days. As treatment is prolonged the incidence of stage 3 tumors increases to nearly 100 per cent.

A mixture of equal parts of 3.5 per cent stilbestrol and 3.5 per cent testosterone propionate in sesame oil was applied topically every day to the right scent glands of twelve animals. Stage 3 tumors appeared bilaterally after a mean of 267 days (range, 166-389) but tended to appear earlier (90 per cent) on the “treated side” and in general were significantly larger than on the opposite side.

Androgens are, in some respects, inhibitors of estrogens. With the flank organ tumor an estrogen and an androgen act together in converting stage 2 to stage 3 tumors. Can another hormonal estrogen inhibitor be substituted successfully for androgen in this respect? Neither progesterone nor deoxycorticosterone acetate can be substituted successfully for androgen in definitive flank organ tumor induction; neither did the addition of these hormones to androgen-estrogen-treated animals prevent the transformation of stage 2 to stage 3 tumors, although the deoxycorticosterone did retard this process.

It has already been emphasized that the application of both male and female hormones is necessary for the production of definitive tumors and that androgen alone can promote the tumor only as far as stage 2. What happens to stage 3 neoplasms when the hormones are withdrawn individually or together? Following simultaneous removal growth ceases, and tumors gradually become distinctly smaller. Histologically these deprived tumors are days. In spite of this extreme range the great majority of latent periods fall within a sufficiently narrow range to permit satisfactory experimentation.

A conspicuous feature of this neoplasm is a very wide range in latent periods for palpability—e.g., in one instance we palpated a tumor after only 53 days of androgen/estrogen treatment, although in other instances no tumors were palpable after 400-500 days. In spite of this extreme range the great majority of latent periods fall within a sufficiently narrow range to permit satisfactory experimentation.
indistinguishable from those in treated animals, except for the absence of mitoses. Upon reintroduction of the hormones growth is resumed. When estrogen alone is withdrawn, growth continues but at a very reduced rate. When androgen alone is withdrawn, growth ceases. Removal of the inducing hormone(s) has a similar effect on the earlier stages. For example, in untreated males, castrated after about 200 days, stage 1 nodules show no changes for the balance of life. The behavior of the fully developed stage 2 tumor is of particular interest in this regard, since it presents the bizarre phenomenon of an androgen-induced, hormone-dependent tumor which will neither regress significantly following androgen withdrawal nor progress following continuous androgen treatment.

Metastasis.—In a group of 487 animals with stage 3 tumors of various sizes, metastases occurred in about 3 per cent; however, all metastases came from animals with large primary tumors. Usually metastases involve the lungs, but occasionally axillary lymph nodes are invaded, and in one instance metastatic growth occurred in the subcutaneous tissue of the right abdominal wall. In approximately 20 per cent of all animals bearing subpinnacular transplants, metastases occur. This is increased to 75 per cent in animals receiving cortisone as well as androgen and estrogen. Each of these percentages could be increased to approximately 100 per cent by limiting the sample to those animals treated for very long periods and containing large active tumors. The incidence of metastases from early generation transplants is essentially the same as that from late passages—e.g., 20th. Metastases repeat the histological characteristics of primary tumors (Fig. 30), sometimes showing areas resembling stage 1 nodules, as well as radiating cords, and frequently areas exhibiting palisading (Fig. 32) and/or whorls (Fig. 33). Metastases require the same hormonal support described for primary lesions and exhibit the same patterns of behavior in tissue culture as does tissue from primary or transplanted tumors.

Transplantation characteristics.—Attempts to transplant fifteen primary tumors were successful only when recipients were androgen-estrogen-treated at the same time or earlier. In no instance was any evidence of autonomous growth apparent. In hormonally treated hosts growth occurred in all transplantation sites employed—i.e., cheek pouch, intraperitoneal, intrathoracic, subpinnacular. Of the original fifteen primary tumors transplanted, eleven were discontinued after a variable number of passages; of the four still being carried the one which has served as donor for the largest amount of experimental material is now (June, 1964) in the 21st serial passage.

Both the latent period for palpability and the subsequent growth rate of transplants are highly variable—e.g., mean latent period of approximately 85 days, with range of 25–175 days. There has been no obvious trend toward shorter latency or increased growth rate in 21 serial passages; neither has there been any visible alteration in the histological appearance between the primary lesion and the 21st passage. Even after back implantation of tumor tissue which had been cultured in vitro in the absence of sex hormones, the subsequent histological appearance, growth rates, and hormonal requirements were unaltered. From a 7th serial transplant roller tube culture were carried for 81 days. From these, Maximow slide cultures were prepared and carried for an additional 9 days. Thirteen mg. of culture material (including plasma clot) were injected into each of two androgen-estrogen-prepared hosts. Both implants grew, and pulmonary metastases were present in each host at autopsy. Tissue deriving from this source has been carried serially in conditioned hosts. Attempts (fifth, eighth, eleventh passages) to grow it in untreated hosts have been consistently unsuccessful. Subsequently, dispersed cell cultures grown for 16 days in the absence of hormones have been successfully back-implanted into hormonally prepared hosts; they did not “take” in untreated hosts.

In a group of eleven hypophysectomized males transplanted growth still required exogenous sex hormone support, as evidenced by complete failure of takes in seven untreated animals but normal growth in three of four treated animals. The effect of progesterone on transplant growth parallels its effect on the growth of primary tumors (i.e., while not preventing growth it tends to retard it). The transplant growth-promoting efficacy of stilbestrol and testosterone propionate individually is essentially the same as described for primary tumors—i.e., estrogen is not as effective as androgen; androgen is only slightly effective.

Samples of tumor tissue from different passages were transplanted to untreated hosts for from 62 to 307 days, after which interval pellets of stilbestrol and testosterone propionate were implanted for a maximum of 350 days. Transplant growth failed except in two hosts receiving treatment after 62 days. Since this experiment actually puts cell survival under the double handicap of severe traumatization of preparing tissue for transplantation as well as hormone deprivation, another experiment was carried out: a series of transplants to hormonally treated animals was permitted to become well established before hormone deprivation. Under these conditions it was possible to initiate active proliferation after as long as 250 days of deprivation.

DISCUSSION

GENERAL CONSIDERATIONS

The terminology used in discussing hormones as carcinogens lacks the precision which is beginning to characterize some of the other areas of chemical carcinogenesis. This reflects our relative lack of fundamental knowledge of hormone action at molecular and cellular levels. The very common implication that “hormone imbalance" constitutes a causal or a permissive agency in carcinogenesis (14, 25, 34, 63) seems, again, to reflect a general lack of specific information. “Hormonal imbalance" must ultimately be defined in terms of specific hormones and their specific actions upon specific targets, the latter being whatever the writer defines—e.g., an organism, an organ, a cell, a part of a cell. Implicit in this concept is the necessity of clarifying the “directness" or the “indirectness" of the actions. Our interpretation is that any stimulus (e.g., hormone or hormonal metabolite,
etc.) acting upon or within a target cell or upon its immediate, as opposed to its remote, environment, in such a manner as to result in neoplastic change within the target, constitutes a direct relationship between the stimulus and the target (42).

Although we have clearly demonstrated that only by combining androgen and estrogen can one secure a definitive stage 3 flank organ tumor, we do not yet comprehend how these hormones, either individually or combined, actually work. We have examined our findings relative to such concepts as "cocarcinogenesis," "initiation and promotion," "synergism," "additive effect," and "potentiation" without increasing our basic understanding of how these hormones are implicated in the induction and subsequent behavior of the chaetepithelioma. Until our knowledge of the mechanisms of action of the two hormones permits a more complete analysis in biochemical terms, perhaps the most satisfactory of the words listed above to symbolize the relationship between androgen and estrogen in the context of this tumor is "synergism"; however, merely saying that these hormones "work together" really is not saying very much. What does "working together" mean? Does it mean, for example, that at the crucial moment a stage 2 tumor cell reaches point X the estrogen can, for the first time, join forces with the androgen in eliciting further (different) effects? If so, what is the nature of point X and what does "joining forces" imply? Do the two hormones act in parallel, in tandem, or do they perhaps unite to form what may be regarded as a unity with new properties? Why do they appear to work as synergists with respect to this and certain other tumors (40) when, in so many other respects, they appear to act as physiological and possibly even as chemical antagonists (6, 20, 50)? Answers to such questions demand knowledge not presently available.

Some insight into tumor-hormone relationships has been gained through studies in vitro. As described more fully elsewhere (2–4), dispersed cell cultures of this tumor flourish in the complete absence of either of the two hormones on which tumor induction and growth are so completely dependent in the animal. It might be argued that this "escape" from hormone dependency is merely part of an irrevocable abnormality acquired by the tumor cells in vitro. Although alterations are known to occur in cultured materials, we believe this argument to be invalid in this instance, since dispersed cell cultures, grown without hormones, resume hormone dependency when transplanted into living hosts. If one subscribes completely to the concept that cancer is a purely cellular phenomenon, then this failure of the "dependent" cells to require hormone(s) for growth in dispersed cell culture becomes enigmatic. It would seem that the cancer cell is able to grow in the host animal only by virtue of the fact that some control mechanism is rendered ineffective by the exogenous hormones. To conclude that, in highly dependent cancers, the hormones are of critical importance for life of the cell is not consistent with the growth of such cells in hormone-free, dispersed, cell cultures.

This tentative interpretation is in harmony with many views that tumors arise as a consequence of indirect action, and one might postulate that the growth of dispersed cells in hormone-free media comes as no surprise, since any check or balance normally controlling growth in the animal is completely absent (61). However, in view of the information obtained from the study of cell cultures it is significant that in organotypic cultures, where the architectural and cellular relationships are reasonably well preserved, a degree of hormone dependency obtains which closely parallels that seen in vivo. Although some tissue may survive for a month or more, mitosis fails unless hormones are incorporated in the culture medium. One conclusion which can be drawn from the organotypic studies of this tumor is that the hormones must be acting at a tissue level obviating perhaps some type of local tumor growth inhibition. This difference in the responsiveness of the flank organ tumor in cell and organ culture is not an isolated finding; a dependent renal carcinoma and a dependent leiomyoma behave similarly (2–4).

Although we have referred and will continue to refer to certain tumors reported from this laboratory as "hormone-dependent," we do not exclude from this term direct hormonal action either in induction or in growth promotion. The concept that "... hormone imbalance per se acts as the specific causative agent in neoplasia" (14) is inadequate to explain some of our observations. We agree that the destruction of a delicately poised balance between the products of one endocrine gland and another may result in oncogenesis; however, we do not have evidence that imbalance per se provokes either the stage 1, the transition of this to stage 2, or the subsequent development of the definitive tumor (stage 3). We do have evidence that the action of the required hormone(s) is essentially direct upon the targets involved—e.g., hair matrix including its immediate environment for stage 1, stage 1 for transformation into stage 2, and stage 2 for continued growth (stage 3). We submit, then, that this concept of dependency be broadened to include not only hormone imbalance per se as causal in neoplasia but to include as well the direct, specific action of a specific sex steroid upon a specific substrate which becomes neoplastic.

In reference to sex hormone-induced tumors in hamsters we interpret complete autonomy as the capacity of the transplanted tumors to establish themselves and grow in castrated hosts. Ideally, the hosts should be adrenalec-

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synthesis, the conversion of ribonucleic acid + amino acid to microsomal ribonucleoprotein (48, 71, 74, 83). It is known that the administration of testosterone to patients with aplastic anemia is followed by an increase in protein synthesis. Consistent with this clinical information is Jacobson's (38) experimental demonstration that in bone marrow homogenates the conversion of glucose-6-phosphate to 6-phospho-d-glyceraldehyde, as a step in the synthesis of nucleic acids, is significantly accelerated in the presence of testosterone. Such evidence suggests that the activity of the messenger ribonucleic acid controlling the transfer of structural information from the genes to the site of protein synthesis in the ribosomes (37, 39, 55) may be regulated in part by this hormone. Such regulation by androgen may be involved in the histogenesis of stage 1 nodules and in their transformation into stage 2 tumors.

Although some evidence does suggest that estrogen may activate specific ribonucleic acid synthesis and in this way affect secondary cellular events in estrogen target organs (71), it seems more probable, at present, that the role of estrogen in the maturation of stage 2 into stage 3 neoplasms is carried on at a different level, possibly through an increased rate of adenosine triphosphate production (76). This suggests that the role of estrogen, unlike that of androgen, may be confined to growth promotion. On the other hand, there is evidence (82) that the carcinogenic action of estrogens may also be connected with their effects on cell membranes, particularly with respect to the liberation from the cells of materials necessary for the formation of connective tissue ground substances. It is suggested that the specificity of action of such steroids on any particular cell depends upon the “ability of the steroid to pack into and remain within some cellular surface membrane.”

In common with others, we have used the term neoplasm and preneoplasm frequently and with apparent confidence. We must state at this juncture that we are in some doubt as to the precise relationship between these two terms in connection with this tumor. Our uncertainty is based upon the observation that a stage 1 nodule is completely normal in all untreated, intact males beyond a certain age, but completely abnormal in all untreated intact females. At the same time these nodules, normal or abnormal, are potentially definitive stage 3 tumors. From this point of view it is justifiable to refer to these as preneoplastic not only in treated animals but in untreated males as well. It is the presence of male sex hormone, either endogenous or exogenous, in relation to age and sex which determines the normality or abnormality of these “preneoplastic” structures.

Specific Considerations

General biology.—The chaetepithelioma possesses superficial similarities to certain other tumors—e.g., storiform neurofibroma (10), dimethylbenzanthracene-induced melanotic tumor (17), neurilemoma; it is not, however, to be confused with any of them or with any of the other neoplasms whose origins have been attributed to hair follicles (22, 26, 33). Although the flank organ shares with many other mammalian scent glands a role in reproduction (60), our present concern is focused on the fact that this scent gland affords a unique opportunity to trace the transition from normalcy to neoplasia through a succession of stages which have been partially characterized both morphologically and endocrinologically.

It is generally accepted that the undifferentiated basal cells of the integument are structurally similar and probably possess identical potentialities for differentiation with the basal cells of the adnexal organs (11, 56). The path of differentiation which the cell follows is determined by extrinsic factors; however, no matter what path is followed, any cell of the stratum germinativum, given adequate opportunity, can undergo keratinous or sebaceous development (53). It is unlikely that the flank organ constitutes an exception to this general rule, even though neither of these types of transformation has been observed in the chaetepithelioma at any stage of its development.

Since the stage 1 nodule appears to arise from the matrix, which gives rise to keratinizing components, the tumor might be expected to contain keratin. In view of the failure of numerous normal and abnormal derivatives of epidermal basal cells to keratinize, however, the failure of the chaetepithelioma to contain keratin is not without parallel. The character of the associated tissues—e.g., mesenchyme, muscle, cartilage—helps determine the degree of differentiation evidenced by the epithermis (52, 81). The presence or extent of differentiation evidenced by the chaetepithelioma, or by any other epidermal structure, is the result of at least two factors: the developmental potential of the epidermal cells themselves, and this may differ in neoplastic as opposed to normal cells; and the nature of the stromal ground substance, including not only its structural and chemical characteristics, but the identity and amount of endogenous and/or exogenous substances, such as hormones, drugs, and various metabolites in its capillary bed.

The most important factor may be the nature of the ground substance immediately adjacent to the epidermal cells, since there is considerable evidence that local differences in the mucopolysaccharides of this substance exist. For example, McLoughlin (52) has demonstrated that different mesenchymes have different effects upon the spreading of epidermal cells. She has correlated these differences with differences in the distribution of acid and neutral mucopolysaccharides in the intercellular materials produced by the mesenchymes, the capacity of extracted cell-free intercellular substance to attract and orient isolated epidermal cells, and the character of basement membranes supplied by these mesenchymes. She has also emphasized that mesenchyme appears to act more or less continuously upon epidermal tissue in contrast to the brief periods of action in classical inductions.

Morphology and morphogenesis.—It is apparent that the dermal sheath is highly involved in the formation of caps. If one accepts the current concept that normal epithelium does not invade normal connective tissue and that such invasion implies malignancy (1), one must then ascribe the property of malignancy to these first matrix cells which escape into the follicular sheath. We question the wisdom of such a rigid criterion. Under certain conditions
of culture it is possible to obtain extensive invasion of epidermis into dermis and even hypodermis. Nevertheless, since growth of such cultures when implanted into either hormone-treated or untreated hosts fails, they are considered to be nonmalignant.  

The relationship between connective tissue and epithelium of the tumor is a striking and characteristic feature. It may be of significance that this interrelationship reflects similar interactions between these tissues of the normal bulb during the catagen phase of the hair cycle. During this transition between active and resting phases the dermal connective tissue sheath of the bulb thickens and becomes somewhat hyalinized to form a characteristic vitreous membrane, the formation of which is followed by a cessation of mitotic activity in the matrix, a pronounced involution of matrix cells, and a rearrangement of the surviving cells to form first a narrow epithelial hair stem and ultimately a thin epithelial plate, or hair germ, between the hair club and the papilla rest. If one imagines a small cluster of matrix cells, isolated from the parent epithelium and embedded in the dermal connective tissue, and if one attempted to repeat these events one would be picturing the formation of a stage 1 tumor. Supporting this concept is the fact that in some instances the inner laminae of a stage 1 corpuscle resemble a vitreous membrane.

In subsequent growth of the tumor, as in the growing (anagen) stage of normal hair follicles (13), proliferation of epithelial cells is accompanied by a migration of epithelium, in more or less straight columns, as the stromal investment thickens (Fig. 29). This analogy between the growth of normal hair and tumor suggests that similar mechanisms may be implicated in the two processes.

The same analogy has been recognized by other investigators (18, 19, 64, 65, 73). Dobson (18) expresses the concept as follows: during the development of basal cell tumors, "... basal cells grow and undergo rudimentary differentiation mainly in the direction of hair follicles, concurrently, differentiation of the connective tissue occurs in relationship to the basal cell masses. In this way a pilary complex, however monstrous in appearance, results. It thus appears that the experimental basal cell epithelioma represents a recapitulation of the embryonic development of the hair. However, this process requires not only epidermal cell anaplasia but also development of an altered stroma."

We suggest that the connective tissue laminae of the stage 1 nodule represent the results of the host's attempt to wall off these displaced matrix cells. If one visualizes a conflict between the connective tissue and the epithelial content of a stage 1 nodule, then the balance of power must be in favor of the latter if a stage 2 tumor is to form. Similarly, the extreme variability in time of appearance and size of stage 2, or even stage 3, tumors might be explained on the basis of the degree to which this walling off process takes place.

This view is in general agreement with that proposed by Orr (59) for other specific examples of epidermal carcinogenesis. The total progressive changes in stromal tissue may appropriately be designated by the term "permutation" proposed by Orr (58). This view does not deny the probability of irreversible genetic alterations within dependent tumor cells as they progress toward and finally achieve full autonomy (24). It does affirm that during the induction period and during the protracted dependent phase the primary site of action of the hormones may be the immediate stromal environment of the epidermal cell, rather than the cell itself. As the dependent tumor develops it must influence the host stroma, behaving in this respect like any other type of solid tumor, eliciting from the host the well known "specific stromal reaction" described first by Bashford et al. (8) and amplified later by Foulds (23). In metastases and transplants, also, the stromal reactions so characteristic of this tumor are interpreted as host responses to the epithelial tumor cells. We wish to emphasize that in order for the chaetepithelioma to grow the "checks and balances" resident in the tissue must be impaired. In living animals and in organotypic cultures this impairment is accomplished by the administered hormones; in diffuse cell culture the hormones are unnecessary, because the organizational pattern of the tissue is absent (4).

A recent review by Willmer (82) has emphasized that in skin cancer there may not be any increase in the frequency of mitosis and that new cells, which would normally move outward toward the free epidermal surface, move into the connective tissue. He has emphasized also that stilbestrol and other estrogens possessing a phenolic ring produce a deficiency in the ground substance or basement membrane which might result in such a migration of epithelial cells in connective tissue formerly inaccessible to them. He suggests that such an abnormality of the ground substance may be the main characteristic of malignancy. Again, in the histogenesis of the estrogen-induced renal carcinoma of the hamster morphological and histochemical alterations of the intertubular stroma preceded the neoplastic tubular alterations (43). Although these stromal changes were originally interpreted as suggesting the possibility of a stromal contribution to the renal carcinoma, it is more probable that they are indicative of an initial stromal influence upon the adjacent tubular epithelium resulting in neoplastic change in the latter. Other examples of an influence of connective tissue upon tumor induction and growth are numerous (28, 43, 57-59, 67, 75).

A study of the embryology of the flank organ (5) has shown that, between the 9th and 10th days, a minute plate of condensed mesenchyme appears under the epidermis at the site of the future scent gland. What factors bring about this mesenchymal condensation, making this localized area of connective tissue different from that of the surrounding skin, and why do they act on this localized area only? Does this altered mesenchyme induce the subsequent epithelial alterations, and if so, how? Is the altered epithelium responsible for the subsequent alterations in the connective tissue? Are these sequential influences of connective tissue upon epithelium and vice versa repeated in the history of the individual adnexa, and if so what factors govern the pattern of increasing localization—i.e., from condensed mesenchyme and epi-
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Fig. 1.—Untreated male hamster, age 540 days. Region of right flank organ shaved; hair over and around left flank organ pushed aside. \( \times 0.6 \).

Fig. 2.—Longitudinal section through left flank organ of 35-day-old, untreated, female hamster. \( \times 36 \).

Fig. 3.—Dorsum of 300-day-old male hamster, given implants subannicularly of one 30-mg. pellet of stilbestrol plus two 30-mg. pellets of testosterone propionate, for 250 days. Region around each flank organ shaved to expose medium-sized chaetepithelioma in each. \( \times 0.6 \).

Fig. 4.—Flank organ club hairs in left side of a 525-day-old female treated with stilbestrol for 268 days. The hairs are in the brief telogen or resting phase of their growth cycle. \( \times 2.5 \).

Fig. 5.—An eccentrically located unpigmented, minute stage 3 chaetepithelioma seen on the dermal surface of the pigmented right flank organ of a 303-day-old female hamster treated with stilbestrol plus testosterone propionate pellets for 253 days. \( \times 5 \).

Fig. 6.—Dorsum of a 462-day-old male hamster given implants of pellets of estradiol plus testosterone propionate for 412 days. The right flank organ includes a small chaetepithelioma weighing 632 mg., the left one a huge tumor weighing 11,221 mg. \( \times 0.6 \).

Fig. 7.—A small, ulcerating stage 3 chaetepithelioma in the left flank organ of a 400-day-old male hamster treated with stilbestrol plus testosterone propionate for 350 days. \( \times 3 \).

Fig. 8.—Sagittal section through the tumor seen in Figure 7. \( \times 7 \).

Fig. 9.—Three clusters of stage 1 and stage 2 chaetepitheliomas (indicated by arrows) at the base of hair follicles among the hypertrophied sebaceous glands of the left flank organ of a 406-day-old male hamster treated with stilbestrol and testosterone propionate for 353 days. \( \times 44 \).

Fig. 10.—A cluster of stage 1 nodules adjacent to the transient zone of a hair follicle in the left flank organ of an untreated 895-day-old male hamster. \( \times 185 \).

Fig. 11.—Preneoplastic cellular primordium of a stage 1 nodule, continuous with the mitotically active matrix of a hair follicle, in the left flank organ of a 100-day-old untreated male hamster. \( \times 700 \).

Fig. 12.—A typical stage 1 nodule, or laminated corpuscle, in the left flank organ of a 450-day-old male given implants of a cortisone pellet for 400 days. (Similar nodules occur in males receiving no cortisone.) A few melanin granules are present in the epithelial cells of the central core. \( \times 730 \).

Fig. 13.—A small stage 2 tumor in the right flank organ of a 226-day-old male implanted with a stilbestrol pellet from the 31st day and a testosterone propionate pellet from the 82d day of life. \( \times 490 \).
Fig. 14.—A tiny cluster of cells (beginning of "cap" formation) external to the basal aperture of the papilla canal in an anagen hair follicle of the left flank organ of an untreated 35-day-old male. ×710.

Fig. 15.—Small cluster of cells ("cap") continuous with the papilla canal of a hair follicle in the left flank organ of an untreated 50-day-old male. ×710.

Fig. 16.—"Cap" at the apex of the papilla canal of a hair follicle in the right flank organ of an untreated, 400-day-old, partial albino, male. ×710.

Fig. 17.—Primordium of a stage 1 nodule, continuous with the matrix of an anagen hair follicle in the right flank organ of an untreated 190-day-old, male hamster. ×710.

Fig. 18.—A stage 1 nodule just beginning to transform into a stage 2 tumor. Slender collagenous spears are radiating outward from a central dense ring partially enclosing a group of epithelial cells, two of which are in mitotic division. The dense ring may be a remnant of the former basement membrane of the hair bulb. From the left flank organ of a 360-day-old male given implants of pellets of stilbestrol and testosterone propionate for 306 days. ×850.
FIG. 19.—Chaetepitheliomas ranging from stage 1 to large stage 2 nodules, in the right flank organ of a 958-day-old male given implants of testosterone propionate pellets for 908 days. ×135.

FIG. 20.—A heavily pigmented tumor incarcerated within the papilla of a hair follicle in the right flank organ of a 330-day-old female given implants of pellets of stilbestrol and testosterone propionate for 180 days and of cortisone for 54 days. ×330.

FIG. 21.—A large, intrafollicular tumor in the right flank organ of a 360-day-old partial albino male treated with stilbestrol and testosterone propionate for 180 days and cortisone for 54 days. ×330.

FIG. 22.—A pigmented, intrafollicular tumor in the left flank organ of a 150-day-old male treated with pellets of stilbestrol, testosterone propionate, and deoxycorticosterone acetate for 100 days. Degenerative changes are apparent in both the nodule and the hair bulb. ×210.

FIG. 23.—A "free" tumor still in contact with the matrix and closing the greatly expanded aperture of the papilla canal of a distorted hair bulb in the right flank organ of a 226-day-old male treated with pellets of stilbestrol for the final 195 days, and of testosterone propionate for the final 144 days, of life. ×220.
FIG. 24.—A small stage 2 nodule which has retained continuity with the matrix of its parent follicle. From the left flank organ of a 150-day-old male given implants of pellets of stilbestrol, testosterone propionate, and deoxycorticosterone acetate for 100 days. X260.

FIG. 25.—A barely palpable stage 3 tumor attached to a cystic hair follicle in the right flank organ of a 360-day-old male hamster given implants of stilbestrol plus testosterone propionate pellets for 306 days. X110.

FIG. 26.—A large stage 2 nodule which has retained continuity with the matrix of its parent follicle. From the left flank organ of a 360-day-old male, partial albino, given implants of pellets of stilbestrol and testosterone propionate for 306 days. X350.

FIG. 27.—Early fusion of a group of stage 1 nodules to form a stage 2 tumor in the right flank organ of a 357-day-old male, partial albino, given implants of pellets of stilbestrol and testosterone propionate for 304 days. X150.
Fig. 28.—Radial arrangement of cords of tumor cells in a primary stage 2 nodule in the left flank organ of a 315-day-old female given implants of pellets of stilbestrol and testosterone propionate for 267 days. ×210.

Fig. 29.—A large stage 2 tumor illustrating the tendency for the tumor cells to radiate outward in centrifugally expanding cords separated by collagenous sheaths which are relatively heavy centrally and thin peripherally. From the left flank organ of a 360-day-old partial albino male treated with stilbestrol and testosterone propionate for 306 days. ×220.

Fig. 30.—Radial arrangement of cords of tumor cells in a pulmonary metastasis from the second serial passage of a subcutaneously transplanted chaetepithelioma in a stilbestrol, testosterone propionate-treated host. ×185.

Fig. 31.—A medium-sized (2,710 mg.) stage 3 chaetepithelioma dissected free from the right flank organ of a 507-day-old male implanted with stilbestrol and testosterone propionate pellets for 406 days. ×4. The lobulation is indicative of a multiple origin from the fusion of many enlarging stage 2 nodules.
Fig. 32.—Nuclear palisading in the first serial subpinnacular passage of a chaetepithelioma. Host treated with stilbestrol and testosterone propionate for 192 days. X185.

Fig. 33.—Arrangement of tumor cells and fibers in whorls in a chaetepithelioma of the right flank organ in a 554-day-old male implanted with pellets of stilbestrol, testosterone propionate, and progesterone for 563 days. X320.

Fig. 34.—A portion of an intraabdominal metastasis from an intraperitoneally transplanted primary chaetepithelioma in a host treated with stilbestrol and testosterone propionate pellets for 150 days. The cords of tumor cells are somewhat broken up and thinned out by the thickened supporting stroma of collagenous fibers. X100.

Fig. 35.—Adjacent pigmented and nonpigmented portions of a chaetepithelioma in the left flank organ of a 416-day-old female treated with stilbestrol and testosterone propionate for 366 days. X180.
Androgen-Estrogen-induced Tumors: I. The Flank Organ (Scent Gland) Chaetepithelioma of the Syrian Hamster

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