SEX HORMONES AND THEIR RELATION TO TUMORS

LEO LOEB, E. L. BURNS, V. SUNTZEFF AND MARIAN MOSKOP
(From the Department of Pathology, Washington University School of Medicine, St. Louis)

In an earlier period it was shown that an ovarian hormone interacting with hereditary factors is responsible for the development of mammary gland carcinoma in mice. It was shown, furthermore, that the number and age of the mice so affected depend in a quantitative manner on the length of time during which the hormone has a chance to act on the recipient tissue, or, expressed differently, on the total quantity of hormone given off during this period. By diminishing this time and the quantity of hormone, the incidence of tumors can be diminished proportionately, and if a certain threshold has been reached the appearance of tumors can be prevented altogether. Strains and families of mice can be separated, which, in accordance with their hereditary characteristics, respond with very unequal readiness to approximately the same quantity of hormone. An analysis of the sexual cycle made it appear probable that, of the two ovarian hormones, it is the follicular hormone which is responsible for this effect.

These investigations have also shown that a relatively small quantity of the hormone, sufficient to induce estrus if acting at regular intervals, is enough to accomplish the carcinomatous transformation of normal mammary gland tissue in that number of individuals which accords with the degree of the hereditary tendency of the family or strain to which they belong. From these investigations it seemed probable that in males mammary gland cancer appears only exceptionally because here the amount of available estrin remains below the threshold. During this period, therefore, the first attempts were made to produce mammary gland carcinoma in male mice (1) by means of transplantation of ovaries into castrated males, and towards the end of this period Murray (2), who also used this method, succeeded in a number of cases.

During this time it was, in addition, shown that besides estrin a second stimulating factor, presumably also of the nature of a hormone, intensifies the carcinomatous transformation of mammary gland tissue, as is indicated by the fact that in breeding mice the cancer rate is higher than in non-breeding mice (3). The degree of difference between the cancer rates of breeding and of virgin mice, however, differs greatly in different strains; in some the difference is small, while in others it is considerable, and this difference seems to be a hereditary characteristic. It is in all probability the intense growth stimulus, acting on the mammary gland during pregnancy, which is responsible for this effect. As Bagg (4) has observed, by inducing pregnancies in rapid succession the tumor rate can be still further increased, and, moreover, as he has

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recently shown, mammary gland carcinoma may appear under these conditions even in low-tumor rate strains.

During this first period it was also observed that after a sufficient amount of ovarian hormone has had a chance to act, during a certain length of time, cancer may develop subsequently long after the action of the hormone has ceased.

More recently, it has been found by Lacassagne (5) that by injecting solutions of estrogenic hormones it is possible to increase, within certain limits, the number of female mice which develop carcinoma of the mammary gland above the index of hereditary tendency characteristic of a certain strain, and, as he has also shown, cancer can be made to develop under these conditions at least as readily in male as in female mice. Furthermore, not only can the appearance of cancer be accelerated in mice belonging to high-tumor strains, but it can be made to appear, although with greater difficulty, even in individuals belonging to low-tumor strains. It is necessary, however, to inject very large doses of estrin into mice in order to increase the incidence of cancer above the heredity index, in contrast to the relatively small doses of estrin which cause the development of the so-called spontaneous mammary gland cancer in these animals.

Recent studies of the developments in this field have given a clearer insight into some of the mechanisms underlying cancerous transformation of normal tissues.

(1) In general, it may be stated that the experimental cancer induced by estrogenic hormones differs from other types of experimental cancer in that in the former it is a normal product of the body which acts as a carcinogenic agent, a substance to which, therefore, the organism is adapted and to which it does not respond with a number of accessory reactions, perhaps of an inflammatory nature, which might tend to obscure the recognition of the essential mechanism of cancer formation.

(2) The development of hormonal cancer is the end stage of a continuous series of growth processes extending over long periods of time, which vary, however, on the average in different strains of mice. Gradually there develop here and there lobules consisting of ducts and acini, lined with relatively large cells, solid in some places and filled with secretion vacuoles in others. If the growth stimuli continue to act, a system of small branching ducts and acini begins to proliferate more actively, mitoses appear, cells and nuclei enlarge—some more, others less—and these structures grow out but, not finding a sufficient space to grow into, coil upon each other in an irregular manner. The center of the cancerous lobule has thus been formed. The same process may proceed with unequal rapidity in different parts of the mammary gland until in the end one can foresee the complete cancerous transformation of the whole organ.

Several important conclusions may be drawn from these observations: (a) The cancerous transformation does not depend upon a gene or chromosome change in a single somatic cell; it does not depend, therefore, upon a somatic mutation in the sense in which this term is used in genetics. Interpretations concerning the biology of cancer, based on such an assumption, are therefore not tenable. As to the differences in the hereditary tendency of
different strains or individuals to undergo cancerous transformation in a
certain tissue, this is a problem distinct from the former one, which concerns
the change taking place in cells and tissues as the result of long continued
stimulation. (b) Inflammatory changes are not an essential or necessary
factor in the origin of cancer. (c) The essential factor in the cancerous
formation is the action of growth stimuli in cooperation with hereditary or
other constitutional factors.

(3) If we compare the growth of the mammary gland in high-tumor and
low-tumor strains under the influence of long continued administration of
large doses of estrin, we are struck by the fact that for four, five, or six months
the growth may be very similar in ovariectomized mice belonging to high- and
low-tumor strains; and in principle the same seems to hold good in non-
ovariectomized female mice as well as in male mice. The growth is relatively
slight for a long time. If, however, we continue the injection of estrin still
longer, differences gradually develop in the growth of the mammary gland
tissue in high- and low-tumor strains. In both growth continues, but in the
former it becomes more extensive and more intense; larger areas of mammary
gland tissue develop, some of which are actively secreting. When this stage
has been reached, the next step, due to the further intensification of growth
processes, leads to the development of cancer.

There are some handicaps which the spurred-on mammary gland tissue
has to overcome in its advance towards cancerous transformation. There is,
first, the tendency of hyaline connective tissue, which often forms around
ducts and also around acini, to constrict and injure these structures. Hyaline
tissue formation exerts this function in both high-tumor and low-tumor strains.
Its influence must not be underrated; but in high-tumor strains the gland tissue
can in many places surmount this obstacle, while in some of the low-tumor
strains the growth energy of the tissue is not sufficient in most places to force
access to a more favorable terrain.

There occurs, secondly, a collapse of fat tissue which is found especially
in poorly nourished animals. In this tissue the mammary gland does not
thrive. This is perhaps one of the reasons why insufficient nourishment of
the mice tends to diminish or delay the formation of tumors in the mammary
gland. These observations furthermore indicate that it is the difference in
the reaction of the mammary gland tissue to specific growth hormones, in
high-tumor and low-tumor strains which is the basis of the difference in the
hereditary tendency to tumor formation in the mammary gland in these
strains. There are various stages in the development of mammary gland
tissue intermediate between the normal relatively resting state and the fully
developed cancerous state. In accordance with their differences in heredi-
tary tendency to cancer formation, various strains of mice differ as to the
intermediate stage which they reach on the average. And, in passing, it
may be remarked that a cystic condition of the gland ducts is not of any
significance in the carcinoma formation in mice. Likewise stagnation and
inspissation of secreted material in the gland ducts and acini seem to injure
these structures and therefore inhibit growth rather than promote cancerous
changes in the tissues.

(4) Estrogenic hormones do not cause cancer formation because they
give origin to a larger amount of tissue, which thus would be a more favorable field of attack for the unknown agent representing the real cause of the cancerous transformation. But the hormone leads to the formation of new tissue because it stimulates the gland, and because it stimulates the gland tissue it causes the cancer formation. Both increase in the amount of tissue and cancerous transformation are therefore the result of the same condition, they are coordinated and not subordinated phenomena. These conclusions are confirmed and extended in other ways, and in particular if we study the effect of estrogenic hormones on vagina and cervix (6).

(5) In vagina and cervix there occurs a rhythmic development of squamous epithelium and keratin at the time of proestrus; but there takes place in addition an irregular down-growth of the epithelium into the underlying connective tissue, which is greater in the sexually mature than in the immature mouse, and which reaches a maximum in old age. In all strains of mice long continued injection of large amounts of estrin increases the length and the frequency of these processes, and we may therefore assume that ovarian hormones are concerned also in their normal development. Now there occurred in one animal spontaneously, and in a considerable number of animals under the influence of long continued injection of large doses of estrin, a further localized down-growth of epithelium into the connective tissue, which assumed microscopically either the character of precancerous proliferation or of an early stage of cancer. In quite a good many animals these changes were multiple; they were found in the vagina, the cervix, and in the beginning of the uterus. If this growth is progressing the proliferating cell strands may break into vessels. Whether such conditions represent an early, still reversible stage of cancer formation or a fully developed irreversible stage seems to us to be not a question of principle. There can be little doubt that reversible changes of this kind do, in the end, become non-reversible, if the stimulation is continued still longer.

There exists, then, a very far-going parallelism between these changes in vagina and cervix and those in the mammary gland. Both are caused by estrin; they may seem to form spontaneously, but in reality are largely due to the action of estrin given off under natural conditions. Both are intensified by very large amounts of estrin. They are cancerous or cancer-like. Also in vagina and cervix there is a steady progression from a slow normal to an abnormal proliferation. But here there can be no doubt that the mass of newly formed tissue is not an essential factor in the carcinomatous or carcinoma-like transformation. Moreover, the hereditary conditions in the proliferations occurring in vagina and cervix differ from those occurring in the mammary gland, at least quantitatively, and perhaps they are entirely different. We may therefore conclude that the hereditary difference between high-tumor and low-tumor strains does not depend upon differences in the elimination of estrin from the body in these two strain types, which would affect mammary gland and vagina and cervix in the same way. This conclusion is supported by our observation that, in high- and low-tumor strains, the effects of estrin are similar during a long period of time, as already stated; the hereditary differences in these strains depend then upon different modes in the response of the tissues to stimulation.
In contrast to the vagina and cervix, carcinoma-like proliferations in the uterus have not been observed by us, nor by others. A down-growth of glands into or through the muscle tissue may occur, or a localized increase in the ramifications of the glands may occasionally be seen, especially near places where inflammatory changes have taken place in the subperitoneal portion of the uterus, and occasionally without these changes; but these proliferations are still far removed from actual carcinomatous transformations. Ulceration in the lining of the uterine cavity is often followed by an ingrowth of squamous epithelium from the adjoining cervix, and in rare cases this regenerating squamous epithelium may send down some irregular processes into the underlying connective tissue, but these are not progressing to cancer-like formations. In addition, there may in some cases apparently occur a limited localized metaplasia of the cylindrical surface epithelium of the uterus, which does not, however, as a rule, lead to the production of typical squamous epithelium, but merely to the formation of a transitional epithelium similar to that occurring in the cervix. We have not been able to induce this metaplasia by injection of estrin into the uterine cavity.

It has been noted by several investigators that long continued injection of estrin in mice may lead to an enlargement of the anterior lobe of the hypophysis, which may be considered in some instances as adenomatous. The opinion has also been expressed that this enlargement is characteristic of high-tumor strains and that it may be connected with the tendency of the latter to the development of mammary gland carcinoma. We have found no such connection, however. The enlargement seems to occur more readily in some strains than in others; but in our observations it was a low-tumor strain, C57, which showed this tendency to the greatest degree.

On the other hand, we were able to show in a different way that an increase in the activity of certain cells in the anterior pituitary, presumably by way of the ovaries, may actually increase the incidence of mammary gland carcinoma in mice without simultaneous injections of estrin. This could be demonstrated in mice which had received multiple transplants of anterior and intermediate lobes of the hypophysis under conditions which made possible the survival of the grafts. In such mice the incidence of mammary gland cancer was definitely increased. The tumor development in this case was preceded by the early formation of much secreting mammary gland tissue.

In contrast to the action of anterior pituitary transplants, neither injection of extracts of anterior pituitary nor injection of corpus luteum preparations seemed to increase cancer formation anywhere in the body, with the possible exception of a sarcoma which followed long continued injections of lutein hormone.

Sarcomas were observed by us to develop in six among the large number of injected mice which we studied. A few instances were also noted by Allen and his collaborators. In five of our cases the sarcoma developed at or near the site of the injections. In one case it formed in the wall of the upper part of the vagina in connective tissue which was kept in a constant state of stimulation by the deposit of hyaline material in the vaginal wall. This led to a continuous ingrowth of the surrounding connective tissue into the hyaline substance and it ended with the formation of a sarcoma.
In three cases, sarcoma formation followed long continued injections of estrin; in three other cases it followed injections of other substances. In a recent instance it was observed by Burns and Suntzeff in a mouse injected, likewise over a long period of time, with a liver extract prepared for the treatment of pernicious anemia. The injection of this substance in mice was irritating. These sarcomas occurred in low-tumor as well as in high-tumor strains. Taking all these, as well as other facts together, there seems to be little doubt that these tumors developed in response to non-specific irritations and were quite different in regard to their etiology from the development of carcinoma or carcinoma-like proliferations in tissues which are specifically stimulated by estrin to undergo growth processes.

(11) If we take into consideration the various data which have now been discussed, it appears most probable that specific growth stimuli, acting over a long period of time, ultimately change the cell equilibrium in such a way that certain substances inducing cell proliferation are propagated in an autokatalytic manner. In addition, such a substance may, in a limited way, also function as an organizer. Either the growth process as such, or a process closely connected with the latter, induces this change, which thus becomes irreversible. As far as is known at present, all the causes of cancer directly or indirectly stimulate growth processes (10). They exert this effect from the beginning or at a later date. Physical as well as chemical agents, which include presumably substances given off by certain metazoic parasites, seem to resemble each other in this respect. The cancerigenic hydrocarbons (11) differ from the other agents merely in a quantitative manner, but not as regards the principle underlying all these actions. It seems probable that estrin causes cancer formation in certain tissues because it acts as a specific and very potent growth stimulus on these tissues, and that its chemical relationship to some cancerigenic hydrocarbons is not the essential factor in this respect.

Normal conditions acting spontaneously and abnormal conditions applied experimentally, both leading to cancer formation, seem to differ in their action only quantitatively. In all cases the end-stage of tissue growth is cancerous transformation if a certain threshold of stimulation, which differs in the case of different tissues and of different species and strains in accordance with hereditary constitutional differences, has been exceeded.

In certain cases, these growth substances, which develop in the stimulated cells apparently, as stated, through processes comparable to autokatalysis, or which may be self-perpetuating within the cells in some other way, may be separable from the cells and induce the production of similar autokatalytic or self-perpetuating growth substances in other cells on which they act, either in the same individual on adjoining tissues or in another individual on tissues analogous to those in which they originated, and here cause a cancerous transformation, either alone or in conjunction with hormonal or other growth stimuli. Such autokatalytic or otherwise self-perpetuating growth substances would then be closely related to intrinsic viruses.

Recent important experiments of Peyton Rous (12) concerning the production of epidermal carcinoma in the rabbit by means of the extrinsic virus of Shope's rabbit papilloma, and perhaps also the findings of Lucké (13) in
the case of a renal adenocarcinoma in frogs, suggest either that such extrinsic viruses also may function as specific growth stimuli, acting in principle in a way similar to hormones, or that there exists, besides the self-perpetuating cancerous tissue state, a second one, in which an extrinsic growth stimulus remains associated with certain tissues and spurs them on to grow without changing their cell state into a permanent self-perpetuating cancerous equilibrium. There exists a third possibility. Rous believes it possible that such an extrinsic virus is the immediate cause of all cancerous growth and that the stimulating and hereditary conditions which have been demonstrated as etiological factors may function merely as preparatory states which enable the virus to become potent. In the case of the hormonal cancer we have found a gradual, step-by-step change from normal to cancerous tissue by way of intermediate, continuous growth processes, which is not limited to one organ, the mammary gland, but may extend to the vagina, cervix, and beginning of the uterus in accordance with the normal growth stimulation which estrin calls forth in these places. We would then have to assume that at some point in these continuous growth processes an extrinsic virus fixes itself to the tissues and from then on becomes responsible for the further growth processes. Either one extrinsic virus would act on the various tissues and might also induce sarcoma formation in certain cases, or different viruses would be responsible for these growth effects. In the large majority of all mice such a virus or such viruses must ultimately be available, because in the majority of mice cancer formation can be induced if the intensity of hormonal stimulation has become sufficiently intense. Furthermore, without sufficient hormonal stimulation the presence of the extrinsic virus would be unable to accomplish the cancerous transformation. Hormones and viruses would then be partners in the production of these cancers. As to the hereditary condition, it would be uncertain whether this is concerned with the readiness with which an intrinsic self-perpetuating growth substance develops, or with the readiness with which the tissue responds to the action of an intrinsic growth substance or to an extrinsic virus. It would be also conceivable that an extrinsic virus was available to a different degree in different strains of mice.

It must be left to further investigations to determine whether an extrinsic virus is active also in hormonal cancer and whether or not two entirely different types of tumors exist, those dependent during their whole course on the presence of a virus, and others due to intracellular changes which are self-perpetuating.

References


