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STEROIDS AND CARCINOGENESIS

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SUMMARY

The unopposed continuous administration of biologically active estrogenic materials produces a wide variety of cancers in experimental animals. Most of these cancers occur in the target tissues for estrogenic activity and appear to be characteristic of the animal rather than the estrogens. In mice and rats the most commonly induced tumor is breast cancer, followed by pituitary chromophobe adenoma, bladder cancer, tumors of the lymphatic system, adrenal tumors, etc. The characteristic tumor occurring in the hamster under these circumstances is clear cell cancer of the kidney. The dog appears to be an exception in that the continuous administration of estrogenic materials does not lead to such tumors but the administration of certain progestogens leads to the production of mammary tumors. Cancers of the cervix occur largely in mice while cancers of the uterine corpus are seen in rabbits. Laboratory primates appear to be remarkably resistant to the carcinogenic effect of steroids, either individually or in combination. In man, there is the suggestion that androgens given for long periods to stimulate erythropoiesis or oral contraceptives in combination with estrogen may be capable of producing benign hepatomas. In man there is also the suggestion that continuous administration of estrogen may produce adenocarcinoma of the corpus uteri and that women who are exposed to very high doses of synthetic estrogen during their intrauterine development have subsequently developed both benign adenosis and clear cell cancer of the vagina.

STEROIDS AND CARCINOGENESIS

The first demonstration that steroids can be carcinogenic in animals is undoubtedly that by Lacassagne[1]. He was able to demonstrate that the administration of estrogen to male mice of the R3 strain with a high incidence of cancer in breeding females was capable of producing mammary cancer in males, which are free of spontaneous mammary cancer in this strain.

There was then an explosive increase in demonstrations that in cancer-susceptible strains of mice the continuous administration of various estrogens led to mammary cancer, cancer of the cervix and, in a few highly specialized strains, to malignancies of the lymphatic system (particularly the thymus), the testis and to either benign or malignant tumors of the adrenal [2]. In addition to this, it was found that animals under long term continuous estrogen therapy frequently developed pituitary adenomas. This latter effect also appears to be strain specific, with certain tumors developing that can best be described as gigantic.

From studies in mice it was apparent that induction of tumors required the continuous administration of estrogenically active material [3]. None of the estrogens employed showed a substantial influence on the organ in which the malignancy developed. This appeared to be a function of the genetic composition of the host and the continuous administration of an appropriate amount of estrogenic activity [2].

These findings were rapidly extended to the rat. However, in contrast to the mice most of the early rat studies were in random bred animals. Noble and his co-workers employed their random bred line of hooded rats [4] and were able to show that the implantation of compressed pellets of estrone led to the development of mammary tumors. These tumors were considered by these authors as being benign. Geschickter[5, 6] considered his estrogen-induced rat tumors as being malignant although Noble and co-workers believed their tumors to be essentially the same as Geschickter's.

Noble and his group noted that two of their rats died early in the course of the estrogen treatment with marked dilatation of the ureters and kidney pelves, whereas two of the animals after 9 months of treatment were found to have their bladders distended with small stones. Dunning and co-workers[7] subsequently reported the strain-specific occurrences of stones and bladder cancers in estrogen-treated Copenhagen rats. When Dunning and her co-workers treated inbred strains of rats with continuous estrogen administration results very similar to those seen in the inbred strains of mice were apparent. The various strains tested showed substantial differences in the percentages developing mammary cancer on continuous estrogenization. As in the mouse, some strains of rats also developed large pituitary adenomas and bladder calculi here associated with bladder cancers. It is of interest to point out that these various strains of rats differed in their threshold for estrogen required for vaginal cornification [8]. Furthermore, occasional animals, as with mice, displayed tumors of the lymphatic system, the adrenal and, I believe, the first occurrence reported of an apparently estrogen-induced hepatoma. A myosarcoma of the uterus was also seen. Unlike the mouse, rats were not reported to have developed cancers of the uterine cervix.

The status of the primary carcinogenic effect of estrogens did not change substantially until they were given for prolonged periods to male Syrian hamsters.

Mathews, Kirkman and Bacon[9] originally demonstrated a primary renal neoplasm induced by the chronic administration of diethylstilbestrol. It soon became apparent that these rodents responded to the continuous administration of estrogen by developing clear cell carcinoma of the kidney rather than the types of tumors previously seen in the other rodents. Kirkman in his fine monograph [10] did an excellent study on the evaluation of various factors on induced carcinogenic effect. The important findings would indicate that the renal carcinogenesis can be inhibited by testosterone, progesterone or desoxycorticosterone. It is of great interest that there is a difference in the latent period as well as in the percentage of tumor inductions with different estrogens. These tumors appear to occur from a direct action of the estrogen on the renal tissue since they can be induced in kidney fragments transplanted into estrogen-treated male hamsters. There is also evidence presented that trauma to the kidney facilitates the induction of such renal tumors. The tumors regress upon the removal of the estrogen stimulus but regrow when the estrogen stimulus is reapplied. Transplants require the presence of the estrogen for their continued growth although many lines, after three or more generations of transplantation, will grow autonomously. The ovary of the female, possibly through the production of progesterone during estrogen administration, inhibits the renal carcinogenic effect of the estrogen. Autonomous tumors show metastases and have been employed to screen compounds for the treatment of renal cancer in man.

Primates seem to be remarkably resistant to the carcinogenic effect of estrogen even after extremely long periods of administration. Indeed we are not aware of any report of significant carcinogenic effect of the administration of estrogen in man where this therapy has been employed for periods of more than 25 years of careful observation. In these instances the administration was generally to postmenopausal osteoporotic women. Mustacchi and Gordan[11] reported that in their group of women to whom the estrogen was administered entirely in a cyclic fashion the incidence of all tumors was less than predicted on the basis of overall incidence and the incidence of tumors of hormonally related tissues was distinctly less than one would predict. In this same regard Wallach and Henneman[12] report followups of up to 25 years of estrogen administration. They do say that there were some malignancies seen during the first 10 years of their study when the estrogens were administered to the patients continuously. None were of the breast and the others will be discussed below. However, in the subsequent 15 years no malignancies were seen, they believe because of the shift in their policy from continuous administration of the estrogen to the cyclic administration of estrogen such as that employed by Mustacchi and Gordan.

The question of the induction of mammary cancer by the continuous administration of estrogen in man was initially believed to have been demonstrated with the reports of the induction of mammary cancer in men with prostatic cancer given continuous estrogen treatment [13, 14]. The generally short period of estrogen treatment and the presence of the prostatic cancer suggest that these are in reality metastatic lesions from the prostate. Indeed, Campbell and Cummins[15] showed such a tumor with positive stain for acid phosphatase. However, it has subsequently been reported by Symmers[16] that mammary cancer has been induced in two male transvestites given continuous estrogen therapy. These latter patients had the estrogen administered topically as well as systemically and also had mammary gland augmentation.

When estrogens first became available they were administered continuously to many laboratory animals including primates. Pfeiffer and Allen[17] attempted to produce cancer in rhesus monkeys and were unable to do so even though they administered either benzpyrene or dibenzanthracene to most of their monkeys in addition to the estrogenic hormone. Three monkeys survived for more than 8 years, 2 of these for more than 10 years. and there was no evidence of pathologic change in mammary glands even though 3 of the monkeys also had estrogen injected directly into the mammary glands. Geschickter and Hartman[18] treated 17 monkeys continuously with no evidence of malignant change although their treatment period extended from 13 months to as long as 7 years, 7 months. There are many other reports of unsuccessful attempts at the induction of cancer in rhesus monkeys but in general the duration of administration is much shorter than with those selected for the above report. Therefore it was of great interest to see the report by Kirschstein *et al.*[19] who treated 5 rhesus monkeys for 2 years with an estrogen-progestogen combination purchased in a pharmacy. After 18 months one of the animals died with an infiltrating ductal carcinoma of the breast with metastases to the axillary and internal mammary lymph nodes, liver and lungs. The remaining monkeys did not show any evidence of malignancy. Drill and his co-workers[20] recently reported treatment of 96 rhesus monkeys for 5 years with 3 dosage levels of 2 different oral contraceptives (Enovid-E and Ovulen) without the development of either mammary tumors or indeed without development of palpable nodules. No mention is made of the development of any other tumors by these animals. These studies are still in progress and we trust that further reports will be available on them.

Arthes and associates[21] reviewed 283 breast cancer patients and 585 matched controls, concluding that the data provided no evidence of the increased use of female hormones, either as estrogens or oral contraceptives, among the cancer cases. Vessey *et al.*[22] reported a case control study of 54 breast cancer patients and 166 patients with benign breast disease, later expanded to include 90 patients with breast cancer and 255 patients with benign breast disease, with 347 matched controls [23]. They could find

no evidence of the increased use of oral contraceptives by the cancer patients and reported that the patients with benign breast disease made less frequent use of oral contraceptives than the controls.

At the WHO Symposium on Pharmacological Models and Contraceptive Development in Geneva in 1973 there was a great deal of discussion of all the reports of malignancies in humans taking either oral contraceptives or estrogens. It seems clearly agreed by both the presenters and the discussers that there is no significant body of evidence to associate the use of oral contraceptives with an increase in malignancies in the individuals taking them properly. There also appears to be a substantial difference of opinion among the regulatory agencies, as represented at the meeting, and the investigators as to our actual ability to transfer data obtained in animals to the situation in man. I myself believe that at the present time there are no animal data which indicate unequivocally that we should discontinue the use of either intermittent estrogen therapy or properly used combination oral contraceptives.

Lemon[24] believed that the "impeded estrogen" estriol is not carcinogenic in rodents and he thinks that it may indeed be employed to protect against development of breast cancer in man. I do not believe that adequate studies of the carcinogenic potential of this estrogen in rodents have as yet been carried out and we will have to await such studies.

It is of great interest that the dog appears to be quite resistant to the carcinogenic effects of estrogen alone. This may be tempered by the fact that adequate amounts of estrogen in most of the canine species are toxic and lead to bone marrow aplasia and deaths possibly too early to allow for the expression of the carcinogenic potential of the estrogen. On the other hand, dogs have a very high incidence of spontaneous mammary cancers, particularly beagles. This potential can be brought to earlier fruition with larger numbers of tumors by the administration of progesterone and other progestins rather than the administration of estrogenic hormone. There has been some speculation that, unlike the rodents and primates which respond to the administration of estrogen with an increased secretion of prolactin, the dog, particularly the beagle, responds with an increase in the secretion of prolactin to the administration of progesterone or progestationally active compounds. This may be responsible for the differences observed in tumorigenic potential of these two types of steroid hormones.

At the WHO symposium in Geneva in 1973 Dr. Berliner[25] illustrated some of the tumors which had been observed by the U.S.A. Food and Drug Administration in their studies on the effect of steroids on beagles. There has been considerable discussion of the tumors induced by various steroids in these dogs and Dr. Berliner points out that these may properly be called masses and have been most common with a chloroethynyl derivative of norethindrone and a chloroethynyl derivative of norgestrel. He makes the statement that neither of the parent compounds has produced a tumorigenic potential in the long range studies even though they have been tested for over six years. On the other hand, the two compounds mentioned do produce tumors in the beagles and they are massive tumors. Very interesting is Dr. Berliner's statement that in their control beagles, presumably untreated, they have not as yet seen spontaneous mammary tumors. Others have reported a high incidence. He remarked that in the discussion at this meeting there is a considerable difference of opinion as to the role these induced tumors play in the decision-making process about the use of the agents in question.

There are no reports of which I am aware that androgenic hormones produce cancer in laboratory animals when administered in high dosage for long periods of time. It was therefore surprising to see the reports on the development of "benign" hepatomas in patients treated with various oral androgens for anemias over long periods of time. There is at least one report of the regression of such a tumor after removal of the androgenic stimulus [26]. It is of further interest that a few apparently similar benign hepatic neoplasms have been reported to be associated with the long term administration of oral contraceptive steroids to women [27].

Again at the WHO contraceptive meeting Dr. B. J. Leonard[28] discussed the great lengths to which they have gone in evaluating the possibility of production of hepatomas following oral contraceptives. The statement was made that no human hepatomas following oral contraceptives have been described and they show an extensive analysis of deaths due to neoplasm of the liver and intrahepatic bile ducts in England and Wales from 1958 through 1971, with no difference in incidence over those years. They carried out very extensive tests in mice, using both inbred mice and their own outbred mice from the Alderly Park colony and said that approximately one third of all chemicals tested produce an increased incidence of liver tumors but that they do not believe that these tests have any clinical significance. Indeed, Leonard says that because the control mechanisms and feedback mechanisms in menstruation are different in women and in rodents it is difficult to see what useful information can be obtained by giving massive doses of those hormones to rodents.

Estrogens administered to guinea pigs do not lead to the induction of cancers, with a few rare exceptions, but to the production of fibrous tumor nodules, particularly in the peritoneum, which sometimes reach massive size. This effect was first reported by Nelson[29]. These observations were greatly expanded by the very thorough and complete work of Lipschütz and his collaborators[30], who studied the activity of practically all the known estrogens and their esters in this regard. They found in general that the continuous administration of small amounts of estrogenic hormones, whether by multiple injection of the free compounds, the less frequent injection of long acting

esters, or the use of pellet implantation, led to tumor production. The fibromatogenic action of estrogens given continuously in small amounts is parallel to the estrogenic activity of the material, providing the threshold level for the fibromatogenic effect is attained.

They found that the fibromatogenic effect could be increased by a variety of injuries to the peritoneum and could be created locally by the intraperitoneal implantation of pellets containing estrogen.

They studied tremendous numbers of steroids and found that in general the biologically active progestins and androgens were effective in preventing the fibromatogenic effects. Unfortunately all this work was done before we were aware of the cytoplasmic estrogen receptors and we do not have any data by which we could compare the large numbers of compounds studied for their ability to inhibit the uptake of estrogen by the cytoplasmic estrogen receptors, if they are indeed involved in this type of tumorigenesis.

The group in Chile also studied the administration of progestational agents to mice and have reported that, particularly in certain strains of mice, the administration of such agents leads to malignant ovarian tumors.

I have been particularly interested in the problem of interaction between steroids and other carcinogenic stimuli. Our recent work has concentrated on the synergism which we have been able to demonstrate between irradiation and estrogen administration on mammary carcinogenesis in the AxC rat. It has been possible for us to demonstrate that estrogen-treated rats exposed to gamma radiation have a significant increase in the number of tumors they develop and a significant decrease in the time to first palpable tumors and the median time to tumor induction. It has further been possible to show that there is an optimal amount of radiation for the greatest number of mammary cancers in this model system. In evaluating the role of many factors in this synergism preliminary evidence would indicate that in the rat the continuous administration of progesterone is capable of inhibiting both the estrogenic carcinogenesis and the synergism with radiation [31]. On the other hand we have now obtained evidence that the removal of the ovary in the host animal to which estrogen is administered leads to the induction of fewer tumors with a greater latent period for carcinogenesis and slower growth rate for the tumors induced in these animals as opposed to their counterparts bearing intact ovaries. In these animals with ovaries removed the continuous administration of progesterone leads to a slightly higher incidence of tumors, but not the level seen in animals with intact ovaries [32].

The tempering role of prolactin levels in this model system is being actively investigated. Final figures are not yet available but it is apparent that the prolactin levels at the time of irradiation do temper the latent period for carcinogenesis, at least for the occurrence of the first tumors. The animals receiving continuous estrogen treatment for a long period frequently develop prolactin-secreting pituitary tumors. These tumors have high levels of prolactin and the animals have a lower incidence of mammary carcinomas.

We have a personal communication from Dr. Shellabarger that the same synergism is evident when the source of radiation is neutrons rather than gamma radiation.

The major demonstration of which I am aware in which steroidal hormones appear to be the causal factor in human carcinogenesis is that made recently by Herbst [33, 34], that the daughters of women given large amounts of synthetic estrogen during their pregnancies have a substantially increased risk of developing clear cell cancers of the vagina and cervix. This therapy was employed for threatened abortions. The initial studies in this area of which we are aware attempted to decrease the fetal loss, a major problem when the mother has diabetes mellitus. The reported success in this area led to the wider use of this therapy for threatened abortion. Fortunately this therapy is no longer being employed for this purpose. Preliminary studies indicate that when the lesions are found early they respond favorably to the topical application of progesterone[35].

There has been no analogous demonstration in an animal model of this phenomenon. However, it is of interest that Dunn and Green[36] reported that after a latent period of two years a single injection of diethylstilbestrol to newborn mice of the BALB/c and C_3Hf strains altered the endocrine balance of these mice and in the females led to the eventual production of vaginal and cervical carcinomas.

However, it has been known for some time that in women carcinomas of the endometrium are associated with signs of estrogenization including pituitary changes, stromal hyperplasia of the ovary, etc. Sommers and Meissner[41] were able to produce changes in the endometrium of rabbits by administration of estrogen. These changes progressed to the development of typical adenocarcinoma of the endometrium. With this background the report by Wallach and Henneman[12] of 4 cases of endometrial carcinoma during the time they were treating their osteoporotic women with continuous administration of estrogen and none in the subsequent years is suggestive of the fact that in man there is the possibility of induction of endometrial carcinoma by the continuous use of unopposed estrogen. This is strengthened by the recent reports of Wilkinson *et al.*[42] and Cutler *et al.*[43] of the induction of endometrial carcinoma by continuous administration of estrogen to women with Turner's syndrome. This latter finding may indicate that there is something about the chromosomal abnormalities associated with Turner's syndrome which makes the individuals more prone to the development of adenocarcinoma of the cervix upon estrogen administration.

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