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Biochemical Studies of Hormone-responsive Mammary Tumors[1]

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Studies in our laboratory have been devoted to the characterization of experimental mammary tumors. We have approached this problem by studying both morphologic and biochemical parameters, with emphasis on achieving correlations, in order to obtain an understanding of this class of malignancies. We have the ability to draw upon the knowledge from clinical studies, such as those conducted by the Cooperative Breast Study Group, in looking for experimental models that simulate the chemotherapeutic sensitivity of the human disease. It is well known that estrogens and androgens, and to a lesser extent progestogens and corticoids, can induce remission of advanced breast cancer. It is essential, therefore, that an understanding be gained of the biochemical alterations accompanying hormonal treatment in an effort to evaluate the mechanism of action of the hormonal agent, as well as to establish the criteria for judging the validity of a particular experimental test system.

For the past several years, we have endeavored to characterize a particular transplantable mammary carcinoma (5–7), R3230AC, an adenocarcinoma with predominantly epithelial elements and a slight amount of interstitial stroma. The growth of this autonomous tumor was inhibited by treatment with estrogen which produced a marked secretory response characterized by extensive vacuolization and distention of acini with a milk-like fluid that stained with Oil Red O. This lactation-like response was shown to be related to the dose of estrogen administered, and it was demonstrated that estrogen treatment produced elevations in the activities of glucose-6-phosphate dehydrogenase, malate dehydrogenase (decarboxylating) and phosphoglucomutase and decreases in glucosephosphate isomerase and α-glycerolphosphate dehydrogenase (4). In addition to the enzyme changes, treatment with estrogen caused a dose-related increase in the amounts of free fatty acids and triglycerides in the neoplasm, although cholesterol levels were not altered (4). Further studies demonstrated that the estrogen-induced elevations in enzyme activities were prevented by concomitant administration of either actinomycin D or actidione, results which strongly suggest that the hormone-induced responses in enzyme activities reflected protein synthesis *de novo* (2, 8).

One of the most intriguing aspects of this biochemical response to estrogen, which resembles the response of the normal mammary gland during pregnancy and lactation, was the accumulation of a milk-like fluid in the neoplasm. This fluid was found to contain lactose, at a concentration of about 2% that found in rat milk, and exhibited a pattern of fatty acids that resembled that found in rat milk. Electrophoresis of the proteins in this milk-like fluid from the tumor revealed the presence of both casein and whey proteins with electrophoretic properties similar to those of the casein and whey proteins in rat milk (1). It was concluded from the above biochemical studies that the inhibition of growth of the R3230AC adenocarcinoma by estrogen treatment was the result of the induction of a metabolic state similar to lactation, and the resultant stimulation of the metabolic pathways resulted in a secretory state rather than cellular proliferation.

In contrast to the effects of estrogen, administration of androgen resulted in a flattening of the epithelial cells, absence of vacuolization, and a dose-related decrease in all enzyme activities studies with the exception of α-glycerolphosphate dehydrogenase (5, 7). Unfortunately, androgen treatment causes only a slight inhibition of tumor growth, suggesting that the decrease in enzyme activities in no way significantly impairs the ability of the neoplasm to grow. It is of interest that recent studies, employing concomitant administration of actinomycin D or actidione to androgen-treated tumor-bearing animals, have demonstrated a partial prevention of the hormone-induced decrease in enzyme activities by these antibiotics. These data indicate that the response to androgens also was dependent on the synthesis of proteins *de novo*.

In cooperation with Dr. Albert Segaloff, we have expanded our studies by examining four lines of the 13762 transplantable dimethylbenz(a)anthracene (DMBA)-induced mammary tumor that were developed by him (9). These neoplasms have been maintained in normal male hosts, androgen-treated hosts (TP-line), normal female hosts, and estrogen-treated hosts (estradiol line). Segaloff has shown that the morphology of these tumors can be influenced by conditioning, since the normal male line appears to contain mostly stroma, whereas the androgen-conditioned neoplastic line is primarily composed of malignant glandular cells. We have investigated the profile of enzyme activities of these four tumor lines and have found that

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the enzyme activities are highest in the normal female line, which is adenocarcinomatous, while the lowest levels of activities were obtained in the normal male line. The growth of the TP-line is stimulated by administration of androgen, and this enhancement of growth is reflected by a concomitant stimulation of certain enzyme activities, the most striking increases being observed with α-glycerolphosphate dehydrogenase activity (3). Experiments have been conducted in which the course of enzyme activity was followed for several weeks during administration of testosterone propionate. These experiments have shown that the androgen-induced increases in glucose-6-phosphate dehydrogenase, malate dehydrogenase (decarboxylating), and α-glycerolphosphate dehydrogenase activities continued to be elevated further during extended treatment with testosterone propionate; these findings suggest a direct induction of enzyme activity by the continued presence of hormone.

It is of interest that administration of estradiol-17β to animals bearing the TP-line neoplasm resulted in an elevation in enzyme activities, somewhat similar to those seen with androgen treatment, but tumor growth was not stimulated. Under these circumstances, treatment with estrogens induced lactation and, as reported above for the R3230AC adenocarcinoma, the stimulation of metabolic activity, as reflected by enzyme activity measurements, resulted in the production of a secretory product and not in cellular proliferation (3, 5).

Most recently, we have initiated a program, in cooperation with Dr. Sidney Weinhouse and Dr. Michael Shimkin, to examine various biochemical parameters of the methylcholanthrene and DMBA-induced mammary tumors after gastric instillation of these carcinogens. Studies have been performed to obtain enzyme profiles on these neoplasms and to determine if correlations exist among the enzyme activities, treating each neoplasm as a separate entity. Several interesting and significant relationships have been demonstrated by regression (least squares) analysis of these data. Table 1 summarizes the level of significance of the correlations between various enzyme activities in these neoplasms. There appear to be several highly significant correlations between certain enzyme activities indicating a degree of genomic control of the metabolism of these neoplasms. Also, the comparison of the methylcholanthrene tumors with the DMBA neoplasms shows that while there are some similarities, there are several differences in correlatable enzyme activities. The differences indicate the existence of subtle metabolic variances between these two carcinogen-induced mammary carcinomas and may indicate differences in their responsiveness to hormonal treatment. It is of interest that examination of the data obtained from enzyme activity measurements of methylcholanthrene-induced neoplasms after ovariectomy revealed a disappearance of significant correlations between glucose-6-phosphate dehydrogenase and malate dehydrogenase and glucose-6-phosphate dehydrogenase and phosphoglucomutase, a result which implies a dependence of malate dehydrogenase and phosphoglucomutase activities on the presence of the ovarian secretions of the host. This result is in agreement with the estrogen-induced responses of these enzymes that were observed in the transplantable mammary tumors.

The data that we have obtained during our studies with various mammary tumors are represented in a schematic manner (Chart 1). The results are compared to the data obtained after the assay of mammary glands from the same tumor-bearing host. The most striking difference between normal and tumor tissues occurred after treatment with androgen. None of the enzyme activities increased in any of the neoplasms when the hosts received testosterone propionate, whereas the mammary glands of these animals were stimulated. Treatment with estrogen produced elevations in several enzyme activities in the mammary neoplasms and also stimulated the quiescent mammary glands. The chart also indicates that the autonomous tumors, R3230AC and 13762, were not affected by ovariectomy, whereas the dependent methylcholanthrene tumor and the normal mammary glands showed decreased enzyme activities in the absence of the endogenous hormonal secretions of the ovary.

The approach to the study of mammary neoplasia used in our laboratory takes advantage of a wealth of knowledge regarding the influence of hormones on the mammary gland. In contrast to work with hepatomas, we can measure the response

Table 1

Methylcholanthrene-induced tumors[a]			Dimethylbenz(a)anthracene-induced tumors[b]			
Y_1	Y_2	P[c]	Y_1	Y_2		P[c]
G6PD	ICD	0.01	G6PD	ME		0.001
G6PD	ME	0.001	G6PD	GPI		0.001
G6PD	GPI	0.01	ME	GPI		0.001
G6PD	PGM	0.01	ME	α-GPD		0.05
ICD	PGM	0.01	GPI	GDH		0.05
ME	PGM	0.02	PGM	DNA	(—)	0.001
GPI	GDH	0.05				

Regression analyses for correlations of enzyme activities/mg DNA in methylcholanthrene- and dimethylbenz(a)anthracene-induced mammary tumors. G6PD, glucose-6-phosphate dehydrogenase; ICD, isocitrate dehydrogenase, decarboxylating; ME, malate dehydrogenase, decarboxylating; GPI, glucosephosphate isomerase; α-GPD, α-glycerolphosphate dehydrogenase; PGM, phosphoglucomutase; GDH, glutamate dehydrogenase.

[a] 22 separate tumors used for regression analysis.

[b] 25 separate tumors used for regression analysis.

[c] P is probability (significant level of correlation) obtained following analysis of 2 dependent variables, Y_1 and Y_2.

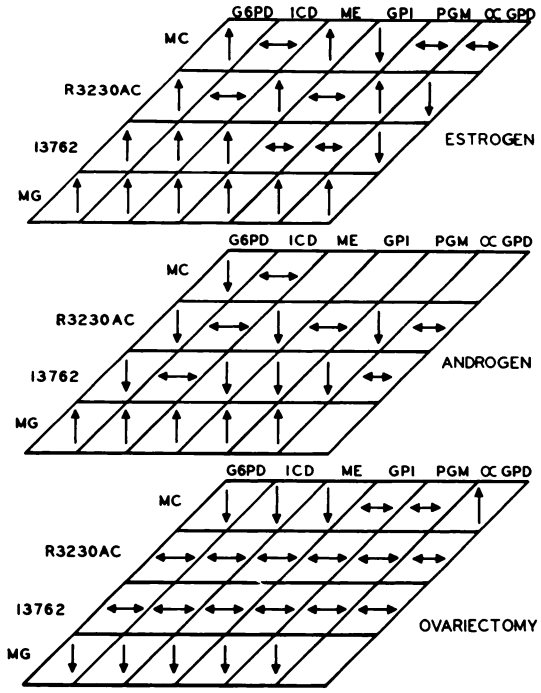

Chart 1. Schematic representation of responses of various tumors and mammary glands after hormonal treatment or ovariectomy. Data are presented for enzyme activities/mg DNA and arrows represent the direction of change in enzyme activity compared to each respective diluent-injected control tissue. MC, methylcholanthrene-induced mammary tumor; R3230AC transplantable mammary adenocarcinoma; 13762, transplantable dimethylbenz(a)anthracene-induced mammary tumor, normal female line; MG, mammary glands from tumor-bearing host; G6PD, glucose-6-phosphate dehydrogenase; ICD, isocitrate dehydrogenase, decarboxylating; ME, malate dehydrogenase, decarboxylating; GPI, glucosephosphate isomerase; PGM, phosphoglucomutase; and α-GPD, α-glycerolphosphate dehydrogenase.

of a mammary tumor to exogenous treatment with various steroids, alone and in combination, the influence of trophic hormones, and the effect of ablation of endocrine organs on the biochemical profile of the abnormal tissue. We are faced with the problem of selection of the normal tissue for comparative purposes, since the metabolic activity of the mammary gland varies strikingly from the quiescent state to the actively lactating state, the enzyme activities in the latter exceeding the enzyme activities of the neoplastic tissues that have been studied. Another serious problem concerns the heterogeneity of the cellular population of mammary tissue compared to the relative homogeneity of the liver. This discussion has presented data that relate to our investigations of hormonally responsive experimental mammary tumors. Unfortunately, approximately 50% of the women with breast cancer show little or no response to hormonal treatment. What are the criteria to be employed in selection of experimental models for the nonhormonally responsive mammary tumors? Hopefully, studies of the human breast cancer tissue, such as those performed by Smith *et al.* (10), and studies along the lines of our studies of the carcinogen-induced experimental neoplasms will reveal patterns of metabolic relationships that are correlatable and indicative of hormonal or nonhormonal responsiveness. Information of this type should lead to a logical selection of chemotherapeutic regimens and to a more sophisticated approach to the selection of new potential therapeutic agents.

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