Biochemical Studies on Mitochondria Isolated From Normal and Neoplastic Tissues of the Mouse Mammary Gland [1, 2]

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SUMMARY—Mitochondria isolated from spontaneous and transplanted mammary adenocarcinomas of two strains of mice were compared, by various biochemical criteria, to mitochondria from mammary glands of midpregnant or hormonally stimulated, cancer-free mice. The specific activities of several mitochondrial enzymes including cytochrome oxidase, α-glycerophosphate oxidase, and succinate dehydrogenase were twofold to threefold lower, whereas the activity of monoamine oxidase was twofold higher in tumor mitochondria. Malate dehydrogenase, adenylate kinase, and NADH oxidase showed similar levels of activity in tumor and midpregnant mammary gland mitochondria. In addition, mitochondrial polypeptide composition was analyzed by electrophoresis on sodium dodecyl sulfate–urea polyacrylamide gels. Midpregnant mammary gland and mammary tumor mitochondria were similar in polypeptide composition; however, several differences were observed. A high-molecular-weight polypeptide, present in midpregnant mammary gland mitochondria was absent from tumor mitochondria. Also, tumor mitochondria contained an additional high-molecular-weight polypeptide not found in the midpregnant mammary gland. There were numerous differences in the relative proportions of many polypeptides common to both tumor and midpregnant mammary gland mitochondria.—J Natl Cancer Inst 56: 65–73, 1976.

Numerous differences between normal and tumor cells have been demonstrated. Those associated with the mitochondria are of particular interest because of the various mitochondrial changes observed in a broad spectrum of neoplastic tissues and also because of the central role played by the mitochondrion in cell metabolism. Differences in tumor mitochondria range from a decrease in the number of mitochondria per tumor cell (*1–4*) to changes in mitochondrial ultrastructural morphology typified by the loss of cristae and changes in the matrix (*5–10*). Of the mitochondria-specific enzymes studied, there is a reduction in activity of adenylate kinase (*7–9, 11, 12*), pyruvate carboxylase (*13*), flavoproteins associated with the mitochondrial electron transport chain, and monoamine oxidase localized in the outer mitochondrial membrane (*7–9*). The activities of some of these enzymes (monoamine oxidase, adenylate kinase, and pyruvate carboxylase) parallel the growth rate and degree of differentiation of the tumors, with the lowest activities associated with the fastest growth rates.

We are currently studying the mitochondria from mammary adenocarcinomas of the mouse. The mouse mammary tumor system is ideally suited to a comprehensive and comparative study of mitochondrial properties in normal and neoplastic cells: Many highly inbred strains of mice are available in which the incidence of mammary cancer ranges from nearly 100 to almost 0%. The viral etiology of mouse mammary cancer is well documented (*14, 15*), and there is compelling evidence that, in the mouse, mammary carcinoma progresses from normal tissue through a preneoplastic stage (*16*). Thus there is a ready supply of spontaneous tumors as opposed to tumor tissues maintained by long serial transplantation. Rapidly growing normal tissue is available from midpregnant animals, and virus-infected versus virus-free tissues and an intermediate stage in neoplastic transformation can be studied.

Our initial studies dealt with the characterization of mitochondria from spontaneous and first-transplant-generation tumors from at least two strains of mice and compared their properties with mitochondria from the mammary glands of midpregnant or hormonally stimulated animals. We were particularly concerned with determining the specific activities of several mitochondrial enzymes known to have altered activities in other neoplastic tissues. In addition, mitochondrial polypeptides were examined by electrophoresis in sodium dodecyl sulfate (SDS)–urea polyacrylamide gels.

MATERIALS AND METHODS

Mammary gland tissue.—Mammary glands were excised from midpregnant (13–16 days) C3H/Crgl, BALB/cCrgl, and BALB/cfC3H/Crgl (CfZ) mice. In addition, mammary glands approximating midpregnant development were obtained from the same strains of virgin mice treated by the sc implantation of E+DCA (0.08 mg 17 β-estradiol, 19.96 mg deoxycorticosterone acetate, 4.96 mg cholesterol) pellets (ICN Pharmaceuticals, Cincinnati, Ohio) for 5 weeks.

Tumors.—Spontaneous mammary adenocarcinomas were obtained from C3H/Crgl or CfZ mice. Transplanted tumors (first transplant generation only, 15–25 mm in diameter) were obtained by the sc implantation of small pieces of spontaneous tumors into syngeneic strains of female mice.

Preparation of mitochondria.—Mammary glands and tumors were minced and suspended in 8–10 volumes of cold sucrose medium (SM) [0.25 M sucrose, 2 mM EDTA, and 50 mM Tris–HCl (pH 7.4)] containing 1% fraction V bovine serum albumin (BSA). The tissues were disrupted in a glass homogenizer by 3–5 strokes of a motor-driven, radially serrated Teflon pestle. The homogenate was filtered through four layers of cheesecloth and centrifuged at 500×*g* for 10 minutes at 4° C. The supernatant was recentrifuged twice at 500×*g* for 10 minutes and once at 8,700×*g* for 15 minutes to obtain a crude mitochondrial pellet. This fraction was washed once by resuspension in 40 ml SM containing 1% BSA and repelleted at 8,700×*g* for 15 minutes. The partially purified mitochondrial pellet was layered onto a 38-ml linear sucrose gradient (1.03–1.71 M sucrose in 2 mM EDTA, 25 mM Tris–HCl (pH 7.4) buffer] and centrifuged for 2 hours at 25,000 rpm in the Beckman SW

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27 rotor at 5° C. At equilibrium, both tumor and midpregnant mammary gland mitochondria were found in a uniform band of density of approximately 1.175 g/ml. When fractions of the gradients were assayed for mitochondria-specific enzyme activity (both cytochrome oxidase and monoamine oxidase), more than 80% of the total activity was associated with the mitochondrial band. The remainder of the activity was found at the top of the gradient. Nonmitochondrial protein was found at the bottom and top of the gradient, well away from the position of the mitochondrial band. There was a twofold increase in the specific activity of mitochondrial enzymes in organelles purified by differential centrifugation alone compared to mitochondria further purified on equilibrium sucrose gradients. However, rebanding of sucrose gradient-purified mitochondria resulted in an increase of specific activity of less than 10%. Therefore, mitochondria prepared in this manner were purified to nearly constant specific activity and were free of any major contaminating nonmitochondrial protein. The mitochondrial band was removed, resuspended in Tris–EDTA buffer at a final sucrose concentration of 0.30 M, and centrifuged at 10,000×g for 20 minutes. The purified mitochondrial pellet was resuspended in a small volume of SM and assayed for enzymatic activity or treated for analysis of polypeptides by gel electrophoresis.

All tissues were homogenized in medium containing 1% BSA to protect mitochondria from disruption during isolation. This method was necessary and effective for mammary gland and mammary tumor mitochondria (*17*) as well as other neoplastic cell mitochondria (*10, 18*). Mitochondria isolated by this method, including sucrose gradient centrifugation, result in highly purified, intact organelles from rat liver, Novikoff hepatoma, and several mammalian cell lines (*7, 8*). A similar method that did not include sucrose gradient centrifugation was used by Mehard et al. (*17*) to obtain intact, respiration-coupled mitochondria from mouse adenocarcinomas and midpregnant and lactating mouse mammary glands.

We separated the mitochondrial membranes from the matrix and intermembrane space proteins by treating the purified mitochondria with the nonionic detergent Lubrol WX followed by washing them in distilled water as described by Schnaitman and Greenawalt (*19*).

Enzyme assays.—We assayed cytochrome oxidase spectrophotometrically by following the oxidation of reduced cytochrome c as described by Smith (*20*); we assayed malate dehydrogenase by observing the oxidation of NADH in the spectrophotometer using the method of Ochoa (*21*); adenylate kinase was tested in a coupled reaction that spectrophotometrically followed the reduction of NADP according to the method of Schnaitman and Greenawalt (*19*); and we assayed NADH oxidase activity by spectrophotometrically observing the oxidation of NADH using cytochrome c as terminal electron acceptor (*22*). The test for monoamine oxidase followed the method of Wurtman and Axelrod (*23*) with ^{14}C-tryptamine (47 mCi/mmole; New England Nuclear Corp., Boston, Mass.) as substrate. We assayed α-glycerophosphate oxidase (the mitochondrial form of α-glycerophosphate dehydrogenase) and succinate dehydrogenase as described by O'Brien and Gethmann (*24*) which spectrophotometrically follows the reduction of *p*-iodonitrotetrazolium violet. All enzymes were assayed for maximum activity and, whenever noted, mitochondria were disrupted by treatment with 0.2 mg of the nonionic detergent Lubrol WX (ICI America, Charlotte, N.C.) per mg mitochondrial protein before the enzymes were assayed.

Protein was determined by the method of Lowry et al. (*25*) with BSA as a standard.

Polypeptide analysis by gel electrophoresis.—The dissociation and electrophoresis of mitochondrial proteins were based on the method described by Maizel et al. (*26*). Samples of highly purified mitochondria (50 μg "Lowry" protein/sample) were disrupted with urea, SDS, and β-mercaptoethanol at a final concentration of 5 M, 0.1%, and 2%, respectively. The mixture was heated at 100° C for 5 minutes and applied directly to the gels after cooling. The gels, set in Plexiglas tubes with 6.0-mm internal diameters, were composed of 10% acrylamide and 0.27% bisacrylamide in 4 M urea, 0.01 M EDTA, 0.1% SDS, 0.1% *N,N,N′,N′*-tetramethylethylenediamine, and 0.05% M sodium phosphate buffer (pH 7.2). Polymerization was catalyzed with 0.5% ammonium persulfate. The gels were electrophoresced at 5 milliampere (mA)/gel for 22 hours in 0.05 M sodium phosphate buffer (pH 7.2) containing 0.1% SDS and 0.01 M EDTA. The gels were fixed, stained with Coomassie blue, and destained by the method of Smith et al. (*27*). The gels were photographed and the negatives scanned with a Joyce–Lobel densitometer. The molecular weights were determined by coelectrophoretic standardization with murine mammary tumor virus (MuMTV) polypeptides (*28*). In all instances where mitochondrial polypeptide profiles were compared, the samples were coelectrophoresced in the same chamber and care was taken to load approximately the same amount of protein on each gel.

RESULTS

The specific activities of several mitochondrial enzymes were compared in many spontaneous and transplanted mammary tumors (table 1). Generally, the activities of all these enzymes did not vary significantly among the approximately 30 tumors obtained from two strains of mice and between spontaneous and transplanted tumors. In addition, tumors from pregnant and nonpregnant animals had similar mitochondrial properties. When the specific activities of these enzymes were compared in mitochondria isolated from the mammary glands of midpregnant or E+DCA-stimulated mice of the C3H or BALB/c strains, no significant variations were detected. However, significant differences were apparent when the enzyme activities of the tumor mitochondria were compared to midpregnant mammary gland mitochondria (table 1). The specific activity of cytochrome oxidase was twofold lower, and α-glycerophosphate oxidase and succinate dehydrogenase were twofold to threefold lower in all tumors as compared to midpregnant mammary gland mitochondria. In contrast, the activity of monoamine oxidase was twofold higher in tumor mitochondria compared to midpregnant mammary gland activity. The activity of malate dehydrogenase was only slightly reduced in tumor mitochondria, whereas the levels of NADH oxidase and adenylate kinase activities were similar for both tumor and midpregnant mammary gland mitochondria. Therefore, there are significant differences as well as similarities in the activities of mitochondrial enzymes in midpregnant mammary glands and mammary tumors.

The analysis of normal and neoplastic tissue mitochondria was extended to a study of mitochondrial

TABLE 1.—*Enzymatic activities of sucrose gradient-purified mitochondria isolated in media containing 1% BSA*

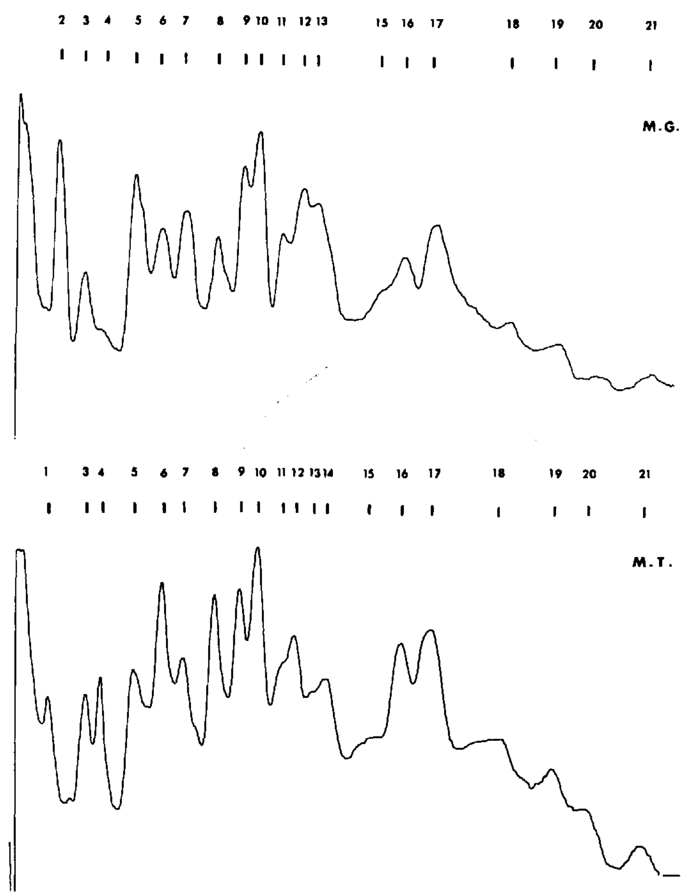
Enzymes	Specific activity (nmoles product formed/min/mg protein) [a]			
	Midpregnant mammary gland	CfZ tumor [b]	C3H tumor [c]	C3H tumor [b]
Cytochrome oxidase[d]	1,365±92.8 (7)	636±75.6 (7)	545±56 (5)	588±45.7 (10)
Monoamine oxidase (^{14}C-tryptamine)	310±27.2 (6)	726±132 (7)	547±68 (5)	705±48.6 (9)
α-Glycerophosphate oxidase	14.1±0.36 (4)	4.34±0.23 (6)	3.87±0.28 (4)	3.13±0.52 (4)
Succinate dehydrogenase	7.4±0.15 (4)	2.72±0.22 (6)	2.21±0.13 (4)	2.8±0.33 (4)
Malate dehydrogenase[d]	1,640±160.6 (6)	1,136±93.1 (6)	918±19 (5)	1,282±151.3 (6)
NADH oxidase[d]	180±17.1 (4)	183±21 (4)	212±15 (4)	160±16 (4)
Adenylate kinase[d]	348±31.3 (5)	327±25.5 (4)	352±20 (4)	320±53.8 (5)

[a] Mean±SE; *numbers in parentheses* = number of individual expts.
[b] Transplanted tumor (first generation).
[c] Spontaneous tumor.
[d] Pretreated with Lubrol WX.

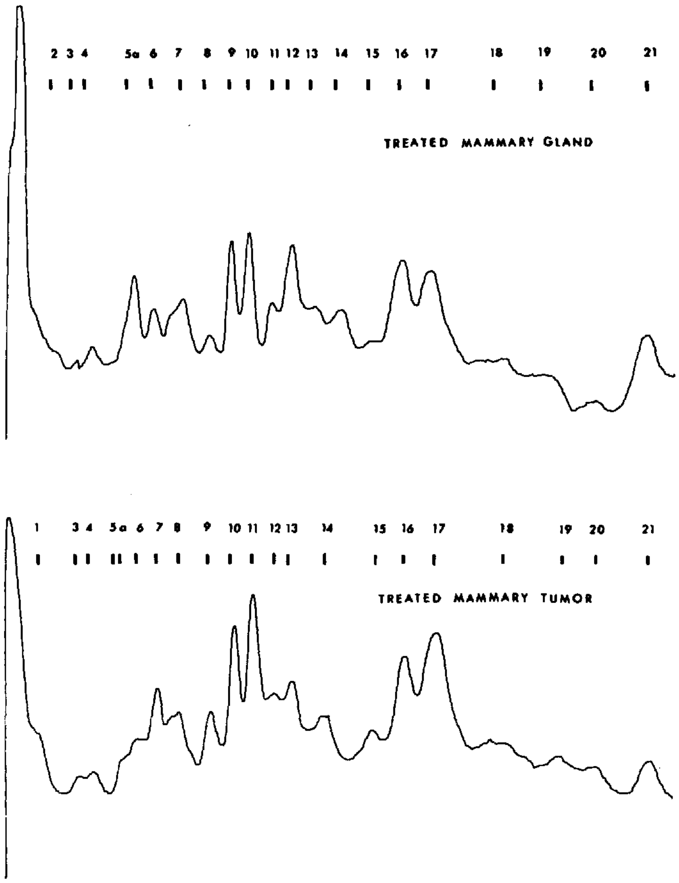
proteins by electrophoresis on SDS–urea polyacrylamide gels. The profiles of mitochondrial polypeptides from three different mammary tumors that were coelectrophoresced were compared in figure 1. There were no quantitative or qualitative differences in the polypeptide patterns of C3H or BALB/cfC3H, spontaneous, or transplanted mammary tumor mitochondria. Generally, the mitochondrial proteins from the over 30 tumors examined showed the same consistent, characteristic electrophoretic patterns. The major polypeptides were present in all preparations in the same relative proportions. Mitochondrial proteins from the mammary glands of midpregnant or E+DCA-stimulated C3H or BALB/c mice also showed consistent, characteristic gel electrophoresis profiles. However, when mammary tumor and midpregnant mammary gland mitochondrial protein coelectrophoretic patterns were compared, significant differences were seen (fig. 2). Polypeptide 1 (87,000 daltons) in the mammary tumor profile was absent in the midpregnant mammary gland profile, but there was an additional polypeptide (84,000 daltons) in the midpregnant mammary gland profile that was absent or greatly reduced in the tumor patterns. Also, polypeptide 14 (41,500 daltons) in the mammary tumor pattern was absent or highly reduced in midpregnant mammary glands (fig. 2, text-fig. 1).

In addition to the presence or absence of major polypeptides in comparisons of midpregnant mammary gland and tumor mitochondria, significant qualitative variations in the electrophoretic profiles were observed (fig. 2, text-fig. 1). The relative proportions of polypeptides 3:4, 5:6:7, and 8:9 were different in midpregnant mammary gland and tumor patterns; this indicated changes in the relative amounts of these proteins in mitochondria from normal and neoplastic tissues. Thus midpregnant mammary gland and mammary tumor mitochondria had many of the same polypeptides, a number of which showed differences in relative amounts.

Mitochondrial membrane components were separated from the matrix and intermembrane space components by treatment of isolated mitochondria with a nonionic detergent (*see* "Materials and Methods"). In figure 3, the coelectrophoretic profiles of polypeptides from untreated (whole) mitochondria were compared to treated mitochondria. Most characteristic mitochondrial polypeptides were still present after detergent treatment. However, in midpregnant mammary gland mitochondria, the high-molecular-weight polypeptide at position #2, present in these tissues but absent in mammary tumor mitochondria, was removed by detergent treatment (fig. 3, text-fig. 2); this indicated that this polypeptide was probably localized in the matrix or intermembrane space of the mitochondrion. Several other polypeptides were also removed by detergent treatment of both midpregnant mammary gland and mammary tumor mitochondria. Polypeptide 5 was greatly reduced in the treated prepara-



TEXT-FIGURE 1.—Densitometer scans of photographs of Coomassie blue-stained gels shown in figure 2.



TEXT-FIGURE 2.—Densitometer scans of photographs of Coomassie blue-stained gels shown in figure 3. Lubrol WX pretreated; midpregnant mammary gland and CfZ spontaneous mammary tumor mitochondrial polypeptides.

tions revealing the presence of another slightly smaller polypeptide (5a) barely detected as a small shoulder in the profiles of the intact preparation (text-figs. 1, 2). Polypeptide 7 in the intact mitochondria profiles consisted of two components by the comparison of treated and untreated patterns (fig. 3, text-figs. 1, 2). Therefore, polypeptides in the size classes 84,000, 64,000, and 61,000 daltons in midpregnant mammary gland mitochondria were removed by Lubrol treatment, an indication that they were probably associated with the matrix and/or membrane space.

DISCUSSION

Unusually high rates of anaerobic and aerobic glycolysis are common metabolic characteristics of many tumors (*29*) including mouse mammary adenocarcinomas (*30*). Many explanations for this phenomenon have been proposed. Warburg (*31, 32*) postulated that a general characteristic of tumors was impaired respiration, such that only the glycolytic pathway was available. However, evidence that some normal tissues exhibit aerobic glycolysis and that there are neoplastic tissues, particularly the minimal deviation Morris hepatomas, which do not show abnormal metabolic activity, disproved Warburg's hypothesis as a general theory of neoplastic transformation (*33*). The fact remains that many tumors, especially those with intermediate-to-fast growth rates, exhibit high glycolytic metabolism, and an acceptable explanation for this phenomenon is lacking. Using the Morris hepatoma as a model system, Criss (*34*) proposed that fast-growing hepatoma cells have adapted to phosphorylating ADP without large need for mitochondrial oxidation. This is due to increased cytoplasmic substrate-level phosphorylating activities and a concomitant decrease in mitochondrial substrate-level phosphorylating activities. In addition, a decrease in the potential of the malate–aspartate and α-glycerophosphate redox shuttle systems and a decrease in potential adenylate homeostasis in the outer mitochondrial compartment, due to decreased adenylate kinase activity, could result in an increased recycling of NADH within the glycolytic pathway. This would explain the decreased need for mitochondrial involvement in the extraction of energy from the oxidation of reduced nucleotides. A severely reduced NADH:NAD$^+$ shuttle resulting from the drastically lowered cytoplasmic α-glycerophosphate dehydrogenase activity of the α-glycerophosphate redox shuttle was measured in over 30 malignant tissues by Boxer and Shonk (*35*). In addition, high glycolytic activity was correlated with low α-glycerophosphate dehydrogenase activity in fast-growing highly dedifferentiated Morris hepatomas (*36*). Kopelovich et al. (*30*) demonstrated high glycolytic activity and low cytoplasmic α-glycerophosphate dehydrogenase activity in mouse mammary adenocarcinomas compared to mammary gland tissue.

We have extended these observations to the mitochondrial component of the α-glycerophosphate redox shuttle—α-glycerophosphate oxidase. Our results show that the specific activity of this enzyme is greatly reduced in mammary tumor mitochondria compared to midpregnant mammary gland mitochondria. Thus both components of the α-glycerophosphate redox shuttle seem impaired in mouse mammary tumors, which would severely limit the ability of these neoplastic cells to transport the NADH generated in glycolysis into the mitochondria where NAD$^+$ could be regenerated via the electron transport chain. This would leave the malate–aspartate shuttle and lactate dehydrogenase as the major routes of NADH oxidation in the tumor cells.

Our results also indicate that the electron transport chain is impaired in mammary tumor mitochondria. The specific activities of cytochrome oxidase and succinate dehydrogenase are reduced twofold to threefold in mammary tumor compared to midpregnant mammary gland mitochondria. Except for cytochrome oxidase, the activity of electron transport components is reduced in the Novikoff hepatoma and hamster melanoma cells (*7, 8*).

The entire electron transport chain of mammary tumors is not abnormal. The activity of NADH oxidase with cytochrome c as the terminal electron acceptor was similar in both midpregnant mammary gland and tumor mitochondria. Also, Mehard et al. (*17*) showed that the coupling of ATP synthesis to electron flow and the cytochrome content of mouse mammary adenocarcinomas were not significantly different compared to lactating mammary gland mitochondria.

The capacity of mouse mammary tumors to control adenylate charge is apparently normal, since our data indicate the specific activity of adenylate kinase is similar in both mammary tumor and midpregnant mammary gland mitochondria. This is in contrast to the reduced levels of adenylate kinase in fast-growing Morris hepatomas (*9, 34*) and the Novikoff hepatoma (*7, 8*). Since liver mitochondrial adenylate kinase is under direct control by the glucose–insulin axis (*34*), abnormalities in the enzyme could be a neoplasia specific to liver and not a general property of all tumors.

A surprising result was the observed twofold higher level of activity of mitochondrial monoamine oxidase in mammary tumors compared to midpregnant mammary glands. The activity of monoamine oxidase in Morris hepatomas (*9*), Novikoff hepatomas (*7*), hamster melanomas (*8*), and simian virus 40-transformed 3T3 cells (*37*) was reduced twofold compared to normal cell mitochondria. Since the biologic function of monoamine oxidase is unknown (*38, 39*), it is difficult to speculate as to why the activity of this enzyme would be significantly reduced in some neoplastic cells and elevated in others. Monoamine oxidase is located in the outer mitochondrial membrane (*40*) and possibly changes in the levels of activity of this enzyme reflect changes in the properties of the outer mitochondrial membrane in neoplastic cells.

In addition to the altered levels of activity of mitochondrial enzymes, we observed specific changes in the number and amount of mitochondrial proteins when analyzed by electrophoresis in SDS–urea polyacrylamide gels. Several mitochondrial polypeptides present in midpregnant mammary gland mitochondria were absent or significantly reduced in mammary tumor mitochondria. Conversely, several polypeptides present in mammary tumor mitochondria were absent or reduced in midpregnant mammary gland mitochondria. Also, the relative proportion of mitochondrial polypeptides compared to one another within the pattern of electrophoresced polypeptide species was considerably different when midpregnant mammary gland and tumor mitochondria were compared. Tumor and midpregnant mammary gland mitochondrial proteins share many similarities in their electrophoretic profiles; however, the amounts of several of the mitochondrial proteins were different.

Isolated mitochondria can be divided into fractions highly enriched for outer membrane, matrix, intermembrane space, and inner membrane components (*19*). In a preliminary experiment, we treated purified mitochondria with the nonionic detergent Lubrol WX which removes matrix and intermembrane space proteins from membrane protein (*19*). In this way, we were able to tentatively assign several characteristic mitochondrial polypeptides identified by gel electrophoresis to the matrix and intermembrane space fraction. We have established the activities of marker enzymes in each of the various subdivisions of the mitochondrion. By subdividing the mitochondrion into various fractions followed by gel electrophoresis, we hope to assign characteristic polypeptides to specific mitochondrial regions and correlate changes in enzyme activity with those in the amount of specific polypeptides. In this way we should be able to distinguish between changes in the amount of a specific enzyme and changes in the structure of a specific enzyme and relate this to the variation in specific activities of tumor and mammary gland mitochondrial enzymes.

The differences in mammary tumor and midpregnant mammary gland mitochondrial enzyme levels and polypeptide compositions are unlikely to arise from contamination of the mitochondrial preparations. Mitochondria were purified to constant enzyme-specific activity, which rules out major protein contamination, and minor contamination could not account for the twofold to threefold differences in mitochondrial enzyme activities we observed. Minor protein contamination might distort the mitochondrial polypeptide gel electrophoresis profiles, but our results indicated a high degree of similarity between tumor and midpregnant mammary gland mitochondrial polypeptides. The significant differences were not the presence or absence of specific polypeptides but the relative amounts of particular polypeptides. Assignment of enzyme activity to one or more specific polypeptides would establish their mitochondrial identity and clarify the question of contamination.

Another question concerns the validity of comparing mitochondria from the midpregnant mammary gland, containing 50–60% parenchymal cells as well as fat, ductal, and myoepithelial cells, with the mammary adenocarcinoma that is predominantly parenchymal. The relative contribution of the various cell types to the mitochondrial population isolated from the more heterogeneous midpregnant mammary gland cannot be satisfactorily answered with whole-gland homogenates. However, enzymatic dissociation of the midpregnant gland yields a fraction containing predominantly parenchymal cells. Mitochondria from this fraction of dissociated midpregnant mammary glands have specific activities for cytochrome oxidase, monoamine oxidase, succinate dehydrogenase, and α-glycerophosphate oxidase similar (80–110%) to the activities observed in mitochondria isolated from whole midpregnant mammary glands (*41*). In addition, the gel electrophoresis profiles were similar if not identical. Also, mitochondria isolated from whole and enzyme-dissociated mammary tumors were almost the same (unpublished observation). Therefore, we are confident that we are comparing similar cell types in this study, using homogenates of mammary tumors and midpregnant mammary glands.

We have shown that mammary tumor and midpregnant mammary gland mitochondria are significantly different. Possibly, alterations in mouse mammary tumor mitochondria are a result of adaptation to glycolytic metabolism. Alternatively, specific alterations in the mitochondria could cause the shift to higher glycolytic rates.

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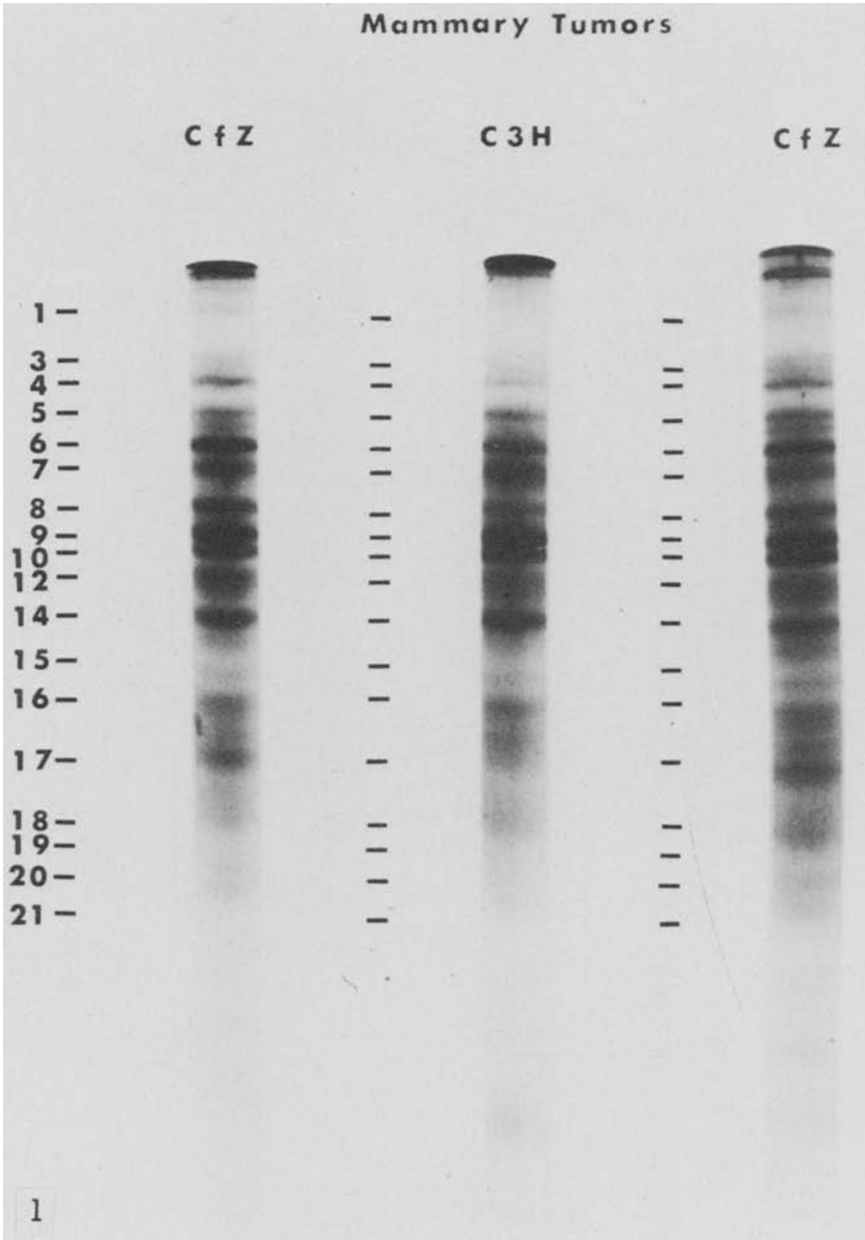

FIGURE 1.—Comparison of coelectrophoretic patterns of mitochondrial polypeptides of 3 mammary adenocarcinomas. *Left* to *right:* CfZ spontaneous tumor; C3H spontaneous tumor; CfZ transplanted tumor (first generation). Mitochondria (50 μg "Lowry" protein/sample) were dissociated by being heated to 100° C for 5 minutes in 0.1% SDS–15 M urea, 0.2% β-mercaptoethanol. Electrophoresis was done at room temperature, 5 mA/gel for 22 hours in 10% acrylamide gel. Gels were stained with Coomassie blue.

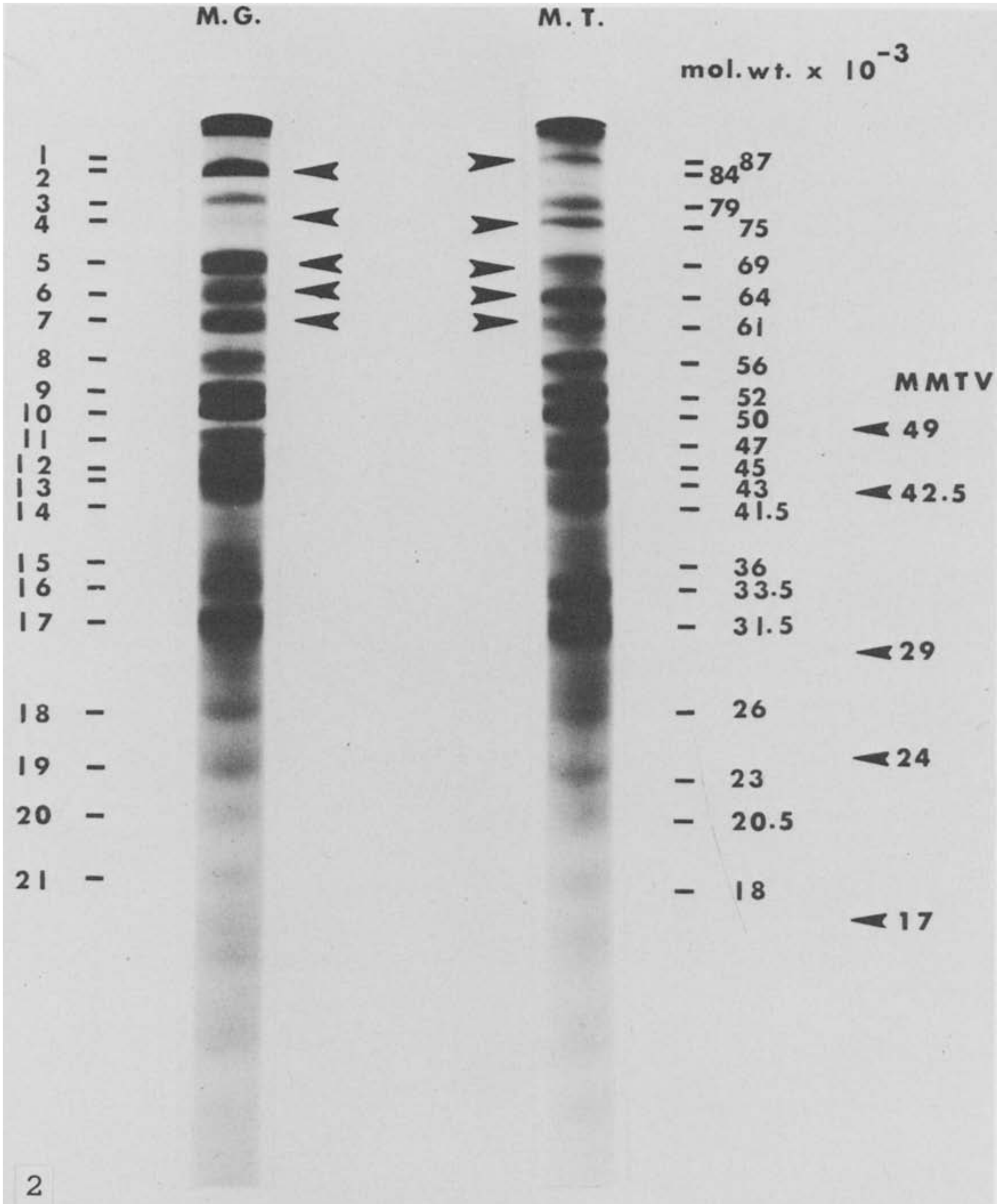

FIGURE 2.—Comparison of coelectrophoretic patterns of mitochondrial polypeptides from midpregnant mammary glands and a spontaneous CfZ mammary tumor. Methods are the same as those described in the legend to figure 1. Positions of MuMTV polypeptides used as molecular weight standards are shown in *right margin*.

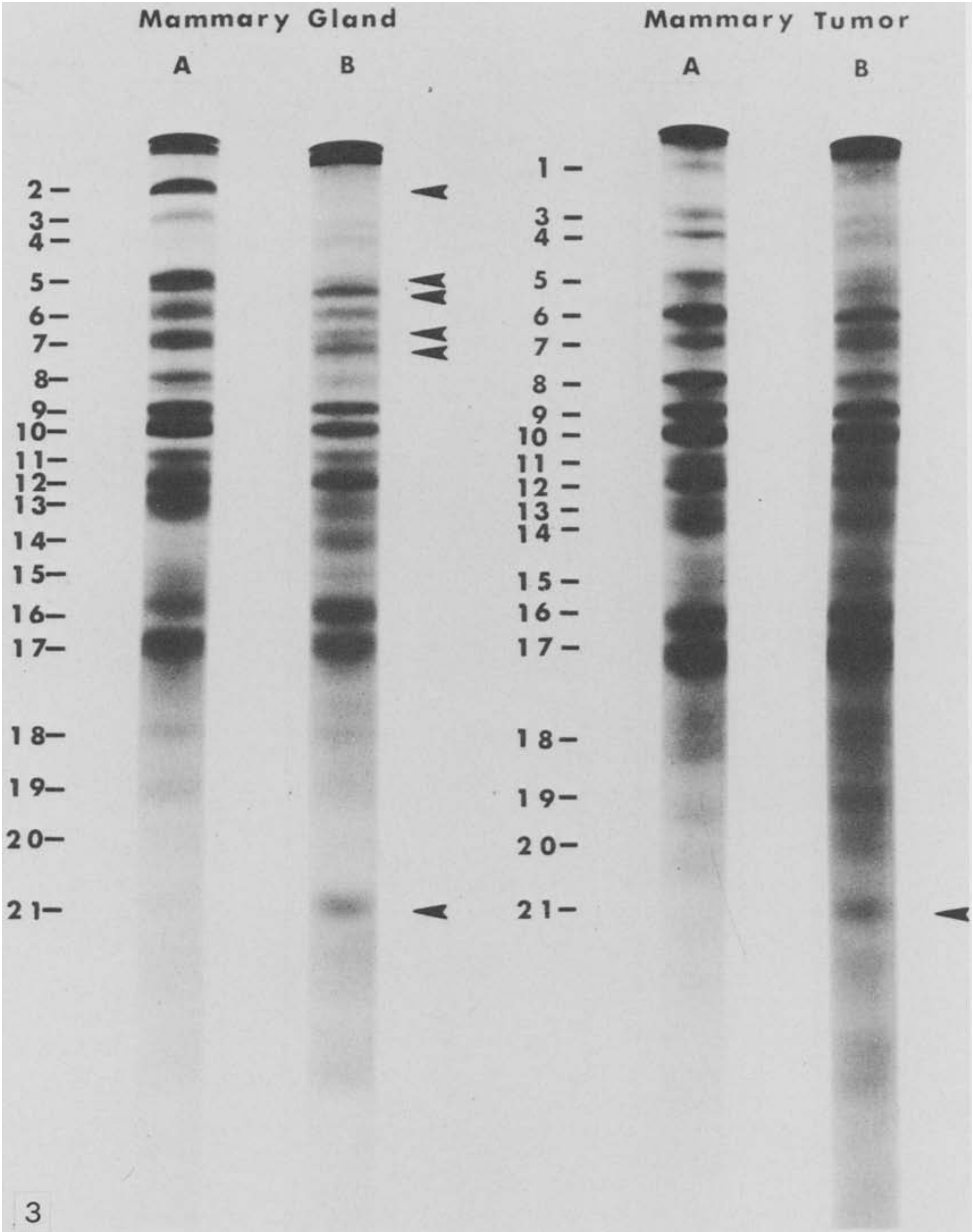

FIGURE 3.—Comparison of coelectrophoretic patterns of mitochondrial polypeptides from E+DCA-stimulated mammary glands: Mammary gland: A) without Lubrol WX pretreatment; B) pretreated with Lubrol WX and a spontaneous CfZ mammary tumor. Mammary tumor: A) without Lubrol WX pretreatment; B) pretreated with Lubrol WX. Electrophoresis conditions are described in the legend to figure 1.