Metabolism of Retinol and Retinoic Acid in N-Methyl-N-nitrosourea-induced Mammary Carcinomas in Rats

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ABSTRACT

This study was conducted to examine the in vivo uptake and metabolism of natural retinoids by N-methyl-N-nitrosourea-induced mammary carcinomas. In this study, endogenous retinol and retinyl esters were present in normal mammary epithelial cells, but were undetectable in N-methyl-N-nitrosourea-induced mammary carcinomas in rats as determined by high-pressure liquid chromatography. No differences were found in plasma levels of retinol, in liver retinyl esters, or total content of vitamin A between tumor-bearing and control animals. Administered labeled retinol was taken up and esterified by normal mammary epithelial cells. Tumor-bearing rats were given injections i.p. of either [3H]retinol or [3H]retinoic acid. Radioactivity increased progressively with time in liver and other tissues except in breast tumor, where the uptake fluctuated over the 8 days after the injection of [3H]retinol; in mammary tumors practically no metabolism of [3H]retinol occurred, while in other tissues extensive esterification was detectable. In contrast, in animals given injections of [3H]retinoic acid, the uptake and metabolism of the label in the breast tumors paralleled with those found in other tissues. Neither the activity of acyl coenzyme A:retinyl acyl transferase nor the activity of retinyl ester hydrolase was altered in the mammary tumor compared to the normal mammary gland. On the other hand, a significant decrease in the retinal oxidase activity was found in tumor tissue compared to normal mammary tissue. Since no esterification of [3H]retinol occurred in vivo despite the presence of acyl coenzyme A:retinyl acyl transferase activity, it is possible that a specific defect in the cellular uptake of retinol may exist in N-methyl-N-nitrosourea-induced mammary carcinomas.

INTRODUCTION

Vitamin A is essential for the maintenance of epithelial tissue differentiation (1). Normal breast is a target tissue for vitamin A; retinol is esterified actively in mammary epithelial cells during lactation, and retinyl esters are secreted in milk (2). Retinyl acetate and 4-hydroxyphenylretinamide were found to have potent antiproliferative activity on normal rat mammary gland development causing decreased ductal branching and end bud proliferation (3). Both natural and synthetic retinoids have been used in large doses in vivo to prevent rat mammary carcinogenesis induced by DMBA (4) or by MNU (5). The appearance of tumors is delayed, and the incidence and severity of tumors are decreased. The addition of large doses of retinyl acetate to the diet of rats bearing established DMBA-induced mammary tumors failed to induce any significant regression in tumors, but retarded tumor growth (5). It has been shown that the growth of transformed human and rat breast cancer cell lines in tissue culture is inhibited by retinoid treatment and that distinct cytoplasmic binding proteins and nuclear receptors for retinoids are present in these cell lines (6–8).

Studies have indicated that levels of retinol, retinyl esters, or retinyl phosphate were greatly decreased in cancer tissues of jejunal mucinous adenocarcinomas (9), human hepatocarcinomas (10), and Morris hepatomas (11) as compared to adjacent normal tissues; the content of vitamin A in breast tumors has not been reported yet. Blood levels of vitamin A have also been found to be lower in patients with various malignancies (12) including breast cancer (13, 14) as compared to control groups. The mechanism for this decrease in blood levels of retinol has not been elucidated, and similar alterations have not been examined in animal models yet.

These studies were undertaken to determine whether a relative vitamin A deficiency was present in MNU-induced mammary tumors as compared to normal mammary gland and to characterize the alterations in retinoid uptake or storage responsible for these alterations.

MATERIALS AND METHODS

Chemicals

HPLC-grade solvents were obtained from the Fisher Scientific Company (Pittsburgh, PA). Standard all-trans-retinol, all-trans-RA, and 13-cis-RA were generous gifts from Dr. W. E. Scott, Hoffman-LaRoche Company (Nutley, NJ). 13-cis-Retinol was synthesized by the reduction of 13-cis-retinal by sodium borohydride (15). Standard retinyl esters were prepared chemically as described earlier (16). Collagenase-type III was obtained from Worthington (Mississauga, Ontario) Palmitoyl coenzyme A, Triton X-100, and 3-[3-cholamidopropyl]dimethyl ammonio]-1-propanesulfonate were purchased from Sigma (St. Louis, MO).

Radioactively Labeled Compounds

All-trans-[11-3H]RA (specific activity, 1.55 Ci/mmol) and all-trans-[10,11-3H2]retinal (specific activity, 3.39 Ci/mmol) were obtained from the chemoprevention program, Chemical and Physical Carcinogenesis Branch, Division of Cancer Cause and Prevention, National Cancer Institute, Bethesda, MD. All-trans-[11,12-3H]retinol (specific activity, 43 Ci/mmol) was purchased from Amersham Corp. (Oakville, Ontario). The purity of the labels was verified routinely by HPLC prior to use.

Animals and Induction of Tumors

Female Sprague-Dawley rats were obtained from Charles River Canada, Inc., at 45 days of age and were fed regular rat chow with water ad libitum; the vitamin A content of the regular rat chow is of the 3.63-mg/kg diet. Mammary tumors were produced by MNU administration as described by Gullino et al. (17); animals received a first intrajugular injection of 5 mg/100 g of MNU (Isopac; Sigma Chemical Co., St. Louis, MO) at 50 days of age with two other injections 4 and 8 wk later. By the 24th wk after the first injection, 92% of the animals had developed tumors which were found to be papillary carcinomas by histology. Animals were used in the following experiments when tumor size reached a diameter of at least 2 cm. Control animals received i.v. injections of 0.9% NaCl solution (MNU vehicle).

Groups of 5 control and 5 tumor-bearing animals were sacrificed to measure the endogenous vitamin A content in plasma and in liver. Plasma retinol was extracted by the method of Thompson (18) after...
the addition of retinylpropionate as an internal standard (19).

Fat-free epithelial and myoepithelial cells were prepared from the normal breast tissues of control animals by the modifications of the technique of Emerman and Pitelka (20). Mammary glands were collected from 4 animals (30 g), placed in a Petri dish at 4°C, and minced extensively with scalpels; the minced tissues were then transferred into siliconized flasks containing (20 mg/g of tissue) DMEM containing 150 mg of collagenase and 0.66 g of bovine serum albumin per 100 ml of medium. The incubation was performed at 37°C in a shaking water bath during 75 min; at the end of the incubation, the medium and tissues were slowly filtered through two layers of cheesecloth, and unbroken tissues were gently disrupted through the cheesecloth with the help of a rubber policeman. The incubation was then pursued for another 35 min at 37°C after the addition of 1 g of Pronase per 100 ml.

The medium was then filtered gently through 600-μm and 150-μm Nitex filters connected in series. The epithelial cells were collected by centrifuging the medium at 400 × g during 10 min and then washed twice with fresh DMEM without enzymes. The cell pellet was collected in 5 ml of DMEM, and an aliquot was utilized to determine cell count in a hemocytometer after staining with crystal violet; the preparation yielded a total number of 2.3 × 10⁶ cells. The viability of the cells was tested by the trypan blue exclusion method and was found to be greater than 90%. The rest of the pellet was frozen at −20°C until analysis on the following day.

Administration of Labeled Compounds to Animals and Extraction of Tissues

Groups of 4 normal rats received an i.p. injection of 50 μCi (1.15 nmol) of [3H]retinol in 50 μl of ethanol. Rats were sacrificed by cervical dislocation after 7 days to allow the administered labeled retinol to equilibrate with the endogenous pool of vitamin A (21). The mammary tissue was removed immediately after sacrifice and processed for the extraction of retinoids as described above.

A group of 10 tumor-bearing animals received an i.p. injection of 10 μCi (0.23 nmol) of [3H]retinol dissolved in 50 μl of ethanol. One animal was sacrificed after 2, 8, 24, and 96 h, and daily during the next 7 days. Another group of 4 tumor-bearing animals received an i.p. injection of 50 μCi (32.3 nmol) of [3H]retinoid acid dissolved in 50 μl of ethanol and were sacrificed 0.5, 1.5, 3.0, or 5.0 h later. The tissues were removed immediately after sacrifice for processing for the extraction of retinoids as described earlier (21).

Vitro Assay of ARAT, REH, and Retinoid Oxidase Activity

A 4% homogenate of normal mammary gland and the tumor tissues was prepared in 0.1 M phosphate buffer (pH 7.4) containing 0.25 M sucrose and 10 μM dithiothreitol. The homogenates were filtered through a cheesecloth. The filtered homogenates were centrifuged at 12,000 × g for 10 min. The resulting supernatants were further centrifuged at 100,000 × g for 60 min. The 100,000 × g supernatants were used as the source of retinal oxidase enzyme. The 100,000 × g pellets were resuspended in the homogenizing buffer and used for the determination of ARAT and REH activities. In the case of liver, kidney, and lungs, the filtered 4% homogenates were subjected to one-step centrifugation at 100,000 × g for 60 min, and the resulting supernatants and the pellets were used for the respective enzyme assays.

ARAT activity was measured according to the incubation conditions described by Ross (2). The REH activity was assayed as reported by Cooper and Olson (22). The retinal oxidase activity was determined using the optimal assay condition described by Bhat et al. (23).

Chromatography

HPLC was performed using a Beckman Model 322 MP programmable liquid chromatograph; a Hitachi Model 100-40 variable wavelength ultraviolet spectrophotometer was utilized to detect retinol and retinyl esters at 325 nm and retinoic acid at 350 nm.

Endogenous Vitamin A in Plasma and in Tissues. The assay of retinol in plasma was performed as described previously (19). The assay of endogenous retinol and retinyl esters present in normal mammary epithelial cells was performed by an initial purification of the lipid extract for retinol and retinyl esters by alumina column chromatography (24). The HPLC separation of retinol and various retinyl esters was performed on a Zorbax TMS column (DuPont Canada, Ontario) using a mobile phase of acetonitrile:water containing 10 mM ammonium acetate (55:45) at a flow rate of 1.2 ml/min during 25 min followed by acetonitrile:water (90:10) at a flow rate of 2.0 ml/min during the next 20 min. The sensitivity of detection of standard retinol in this system was 5 pmol when the detector was set at 0.01 Absolute Units in Full Scale.

Metabolism of [3H]Retinol. The separation of polar metabolites of [3H]retinol in tumor-bearing animals was performed on a 10-μm Partisol-ODS-2 HPLC column (Whatman, Inc., Clifton, NJ) using a mobile phase of methanol:water (80:20) at a flow rate of 1.2 ml/min for 30 min and of 2.5 ml/min for the following 20 min as described earlier (21). For screening purposes, retinyl esters were quantified by alumina column chromatography. The HPLC separation of retinyl esters was performed on a 5-μm Ultrasphere-ODS column (Beckman Instruments, Toronto, Ontario) with a mobile phase of methanol:water (98:2) at a flow rate of 1.5 ml/min for the first 62 min and of 2.0 ml/min for the following 24 min as described earlier (16).

Metabolism of [3H]Retinal. The separation of [3H]retinoic acid metabolites were performed after treatment of tissue extracts with diazomethane in ether for 5 to 10 min; the yield of methyl esters of retinoic acid was almost 100%. The methylretinoids were separated using two reverse-phase 5-μm ODS-1 and 10-μm ODS-2 columns (Whatman, Inc.) connected in series and eluted with a mobile phase of methanol:water (82:14) with a flow rate of 1.2 ml/min.

Products of Enzymatic Assays. (a) For ARAT activity after incubation with [3H]retinol, enzyme proteins, and palmitoyl coenzyme A for 10 min, the reaction products were extracted with 400 μl of butanol:acetonitrile (1:1), mixed well, and centrifuged at 1000 × g for 15 min. Twenty μl of the supernatant were directly injected onto Partisil ODS-2 (Whatman, Clifton, NJ) column. Retinol and retinylpalmitate (the product) were separated in the ODS-2 column by eluting with a mixture of acetonitrile:dichloromethane:methanol (70:20:10) at a flow rate of 1.2 ml/min. In this system, retinol and retinylpalmitate had retention times of 4 and 16 min, respectively.

(b) For REH activity, after incubation with retinylpalmitate, enzyme protein, and detergents, the reaction was quenched by the addition of 0.5 ml of ethanol, and retinol was extracted twice with 2 ml of hexane. The extract was dried under a gentle stream of nitrogen, the residue was redissolved in 500 μl of isopropanol, and 20 μl were injected onto a Partisil ODS-2 column. Retinol was determined using the HPLC system described above.

(c) For retinal oxidase, after incubation with [3H]retinol, enzyme protein, and the cofactors the reaction was quenched by cooling at 4°C. The reaction products were extracted with 400 μl of butanol:acetonitrile (1:1) as described above. Twenty μl of aliquot were injected onto Partisil ODS-1 column. Retinoic acid (the product) was separated by elution with acetonitrile:water (55:45) containing 10 mM ammonium acetate, at a flow rate of 1.2 ml/min. In this system retinoic acid had a retention time of 9 to 10 min.

Fractions from the columns were counted for radioactivity after addition of 7.5 ml of scintillant (PCS; Amersham) in a Beckman Model LS-230 liquid scintillation counter with an efficiency of 30% for tritium.

RESULTS

Content of Endogenous Retinol and Retinyl Esters in Normal Breast, in Breast Tumors, in Plasma, and in Liver Tissues. In the fat-free preparation of epithelial cells from the normal mammary glands, retinol and three peaks of retinyl esters were detected by HPLC analysis (Fig. 1). One peak coeluting with all-trans-retinol (Peak 1) amounts to 78.3 pmol/10⁶ cells; retinyl ester peaks coeluting with retinyl palmitate and oleate (Peaks 11 and 12), retinyl stearate (Peak 14), and retinyl arachidonate (Peak 15) amounted to 283.1, 97.4, and 260.3 pmol/10⁶ cells, respectively.
the peak incorporation occurred earlier, between 3 and 8 h after the injection. In the breast tumors, the uptake was found to fluctuate during the period of 8 days after the injection of the label.

The lipid extracts of breast tumors were processed to examine the nature of polar metabolites and retinyl esters formed from $[^3H]$retinol using the two HPLC systems described in "Materials and Methods." Fig. 2A illustrates the polar metabolites identified in a breast tumor 72 h after the injection of $[^3H]$retinol. It can be seen that the vast majority of the label was found to elute in the position of all-trans-retinol; other small peaks coeluted with standard 13-cis-retinol and phosphorylated metabolites of retinol but were not characterized further. Fig.

Table 2 Incorporation of radioactivity into various tissues of tumor-bearing rats given injections of 10,000 c.p.m. of $[^3H]$retinol

<table>
<thead>
<tr>
<th>Time</th>
<th>Liver</th>
<th>Intestine</th>
<th>Kidney</th>
<th>Breast tumor</th>
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<tr>
<td>3 h</td>
<td>170.0</td>
<td>21.1</td>
<td>33.4</td>
<td>15.1</td>
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<td>8 h</td>
<td>154.0</td>
<td>15.3</td>
<td>61.4</td>
<td>10.1</td>
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<tr>
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<td>250.7</td>
<td>12.2</td>
<td>41.2</td>
<td>25.6</td>
</tr>
<tr>
<td>2 days</td>
<td>516.1</td>
<td>29.7</td>
<td>6.2</td>
<td></td>
</tr>
<tr>
<td>3 days</td>
<td>717.6</td>
<td>13.5</td>
<td>17.5</td>
<td>16.2</td>
</tr>
<tr>
<td>4 days</td>
<td>1437.6</td>
<td>8.1</td>
<td>13.3</td>
<td>4.6</td>
</tr>
<tr>
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<td>3.6</td>
<td>4.7</td>
<td>2.0</td>
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<td>6 days</td>
<td>872.3</td>
<td>7.6</td>
<td>10.8</td>
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<td>7 days</td>
<td>439.1</td>
<td>3.1</td>
<td>4.3</td>
<td>1.7</td>
</tr>
<tr>
<td>8 days</td>
<td>401.4</td>
<td>4.9</td>
<td>2.7</td>
<td>12.8</td>
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</table>

In breast tumors, no endogenous retinol or retinyl esters could be detected even in tumors as large as 10 g. Plasma levels of retinol were similar between a group of 5 tumor-bearing rats (19.3 ± 5.0 µg per 100 ml; mean ± SD) and a group of 5 control rats (20.5 ± 3.4 µg per 100 ml). The total liver content of vitamin A was not different between control and tumor-bearing rats (Table 1); it can also be seen in Table 1 that the content of all-trans-retinol in the liver and the distribution of the various retinyl esters were similar between control and tumor-bearing animals.

Uptake and Metabolism of $[^3H]$Retinol in Normal Mammary Epithelial Cells and in Breast Tumor-Bearing Animals. The incorporation of radioactivity from $[^3H]$retinol into normal mammary epithelial cells, expressed as dpm/10^6 cells ± SD (n = number of determinations), was 1883 ± 534 (n = 4). The pooled lipid extract after alumina chromatography and HPLC separation showed 12% and 88% of recovered radioactivity as retinol and retinyl esters. The incorporation of radioactivity from $[^3H]$retinol into various tissues of breast tumor-bearing animals is presented in Table 2. In liver tissues, the uptake increased with time, and the peak of incorporation was reached 4 days after the injection of the label; in intestine and kidney,
In contrast, in the lipid extracts of the intestine of an animal sacrificed 48 h after the injection of the \[^{3}H\]retinol, 64.7% of the radioactivity eluted in the retinyl ester fraction on the alumina column; in the liver of breast tumor-bearing animals, the percentage of radioactivity distributed in the retinyl esters fraction increased progressively from 8.6% at 3 h to 17.0% at 24 h and to a maximum of 69.1% 96 h after the injection. HPLC analysis of retinyl esters present in the liver 96 h after injection of the label revealed that 76% of the total radioactivity was present in the palmitate, 12.6% in the stearate, 3.2% in the linoleate, 2.2% in the palmitoleate, and 1.7% each in the laurate and heptadecanoate esters of retinol.

Uptake and Metabolism of \[^{3}H\]RA in Breast Tumor-bearing Animals. The incorporation of radioactivity from \[^{3}H\]RA into various tissues of breast tumor-bearing animals is shown in Fig. 3. In all four tissues examined, the uptake was higher at the 0.5-h time point and decreased rapidly afterwards. The lipid extracts of these tissues were methylated and analyzed by HPLC as described in “Materials and Methods” to examine the metabolism of \[^{3}H\]RA. Fig. 4 illustrates the HPLC separation of the extract of a breast tumor of an animal 90 min after the injection of \[^{3}H\]RA. At that time, only 6.1% of the radioactivity coeluted with the methyl retinoate standard; the major fraction of the radioactivity (53%) eluted as several polar metabolite peaks with retention times up to 30 min. The radioactivity recovered as the methylster of all-trans-RA at various times after the injection of the label is shown in Fig. 5. It can be seen that \[^{3}H\]RA was metabolized to unidentified polar metabolites as rapidly in breast tumors as in other tissues examined; in fact in breast tumors and in blood, no more all-trans-[^{3}H]methylretinoate was detectable 5 h after the injection of the label.

ARAT, REH, and Retinal Oxidase Activities in Normal and Tumor-bearing Tissues. ARAT activity, expressed as pmol of retinol esterified/mg of protein/10 min ± SD (n = number of tissues assayed), in the normal mammary gland (95 ± 18, n = 6) and mammary tumor (131 ± 38, n = 6), indicated no significant difference between the two groups. No difference was also observed in the ARAT activities between normal liver (344 ± 74, n = 6) and tumor-bearing liver (320 ± 72, n = 6). REH activity expressed as nmol of retinol formed/mg of protein/h was not significantly different between normal mammary gland (0.60 ± 0.15, n = 6) and mammary tumors (0.67 ± 0.06, n = 5). On the other hand, a significant decrease in the REH activity was found in the liver of tumor-bearing rats (3.14 ± 0.76, n = 6) compared to normal liver (6.67 ± 1.73, n = 6). Retinal oxidase activities in tissues of normal and tumor-bearing rats are presented in Table 3. Although no significant differences in the retinal oxidase activities were found between normal and tumor-bearing kidneys and lungs, a significant decrease in the activity was noted in the mammary tumors compared to normal mammary glands.

DISCUSSION

Our study demonstrates clearly for the first time the presence of retinol in and at least three retinyl esters in nonlactating mammary gland epithelial cells; the retinyl esters present differ qualitatively from those found in rat milk by Ross (2) suggesting that the hormonal milieu modulates retinol esterification in the mammary gland. No endogenous retinol or retinyl esters could be detected in MNU-induced mammary tumors which contained up to 10 times the number of cells present in the fat-free normal mammary gland epithelial cell preparation. We have not measured vitamin A content in normal mammary tissue adjacent to cancer tissue; however, our findings are indicative that a relative localized vitamin A deficiency is present in MNU-induced mammary cancers in rats. This observation is in accordance with similar findings in other human and animal tumors (11, 10, 24).

The relative vitamin A deficiency is limited to the breast cancer in this model, since we have found that plasma and liver levels of endogenous vitamin A were normal in the tumor-bearing animals. These findings diverge from the report of decreased plasma and liver levels of vitamin A in cancer patients (13, 12); it is unclear yet whether these alterations in vitamin A levels are secondary to a chronic debilitated state in terminally ill cancer patients which our tumor-bearing rats had not reached yet.

The incorporation and metabolism of retinol mainly to various retinyl esters in livers of the breast tumor-bearing animal were very similar to what we have previously reported for control vitamin A-sufficient animals (21) up to 24 h after the injection of the label. An active metabolism of retinol mainly to retinyl esters was also found to be present in intestine, kidney, and normal mammary gland.

In contrast, in the breast tumors, the small amount of polar metabolites and the absence of retinyl ester formation from the \[^{3}H\]retinol indicated that some abnormality in uptake or metabolism of retinol was present. Retinoic acid on the other hand was found to be taken up and metabolized as actively by the breast tumors as by other tissues examined. To our knowledge, no previous study of the metabolism of retinoids in tumor tissues has been reported yet. Moon et al. (3) studied liver and breast levels of retinoids in groups of rats on diets containing either no retinoid, retinyl acetate, or 4-hydroxyphenylretinamide during 6 mo after MNU or vehicle administration; retinyl acetate and 4-hydroxyphenylretinamide metabolites (unidentified) accumulated in breast tissues without tumors in a dose-dependent manner, but it is impossible to distinguish whether the metabolism occurred in the epithelial or in the fat tissues of the mammary glands. In that study, no data were provided on whether retinoids could be found in tumors which occurred in MNU-treated animals despite retinoid supplementation.

We examined whether the failure to metabolize retinol to retinyl esters in the breast tumors was associated with a lack of ARAT activity. Our results indicated no change in the ARAT activity between normal and tumor tissues, suggesting that the lack of retinol metabolism was due to a lack of retinol uptake.
Fig. 4. HPLC separation of metabolites found in MNU-induced rat mammary tumor (C) 90 min after the i.p. injection of 50 µCi of all-trans-[3H]RA. The lipid extract was treated with diazomethane prior to separation using the HPLC conditions described in "Materials and Methods." All-trans-[3H]RA was also added to a frozen liver tissue (O) as control prior to extraction, methylation, and separation by HPLC.

Fig. 5. Radioactivity recovered as all-trans-methyl retinoate in various tissues of tumor-bearing rats given injections of 50 µCi of [3H]RA. O, liver; D, kidney; , mammary tumor; A, blood.

Table 3 Retinal oxidase activity in tissues of normal and tumor-bearing rats

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Normal</th>
<th>Tumor bearing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidneys</td>
<td>1.61 ± 0.76</td>
<td>1.06 ± 0.47</td>
</tr>
<tr>
<td>Lungs</td>
<td>0.18 ± 0.15</td>
<td>0.19 ± 0.12</td>
</tr>
<tr>
<td>Mammary glands</td>
<td>0.63 ± 0.17</td>
<td>0.09 ± 0.07</td>
</tr>
</tbody>
</table>

*Mean ± SD.
*n = number of rats/group.
*P < 0.01.

lack of retinyl esters in tumors clearly is not due to an absence of ARAT activity. The presence of ARAT activity in DMBA-induced transplantable mammary tumors in rats has been reported by Ball et al. (25). The other possibility of increased utilization or catabolism of vitamin A may exist in breast tumor tissue. This was partly investigated by studying the REH and retinal oxidase activity. Like ARAT activity, REH activity was also not altered in the tumor tissue. However, a significant decrease in the retinal oxidase activity in the tumor tissue (Table 3) indicated that the utilization of retinol via retinoic acid is impaired. This might have been caused by the low availability of retinol.

These observations further strongly suggest that the uptake of retinol from the blood could be defective in MNU-induced mammary carcinomas. The unmetabolized [3H]retinol found in the tumors could be present in the blood supply of these richly vascularized tumors, and this would explain the erratic uptake observed. The delivery of retinol from serum RBP to target tissues has been claimed to involve cell surface receptors for RBP in bovine and human pigment epithelial cells (25), monkey intestinal mucosal cells (26), chicken and rat gonadal cells (27), and corneal epithelial cells (28). However, it remains to be shown that a cell surface receptor for the retinol-RBP complex exists in the mammary gland in vivo and that it is mediating the uptake of retinol in the mammary epithelial cells.

It will be of interest to verify if a localized vitamin A deficiency is present in other cancer models in animals as well as in humans. Although most emphasis on the relation between vitamin A and cancer has been placed on dietary intake of carotenoids or vitamin A and on plasma levels of vitamin A (12), we believe that it is also important to examine possible alterations of the delivery or the action of vitamin A at the target tissue level where vitamin A is essential to maintain differentiation. Localized deficiencies could result from any abnormalities including defects of RBP-retinol cell surface receptor, metabolism to active or inactive metabolites such as anhydroretinol (29), alterations of intracellular retinoid binding proteins, nuclear receptors, or any other step involved in vitamin A action.

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