correlated with tumor size ($r = .842$). When tumor growth was inhibited with either local irradiation or administration of 5-fluorouracil, the increase in cyclic GMP excretion was also delayed. Furthermore, within 1 day of tumor excision cyclic GMP excretion returned to control values. No alterations in cyclic AMP excretion were observed under any of the conditions examined.

The increased excretion of cyclic GMP may result directly from the release or secretion of cyclic GMP by the tumor or an effect of the tumor on other tissue or tissues. Thus, the tissue source for the increased urinary cyclic GMP has not been established, and additional studies are needed. Nevertheless, the correlation of cyclic GMP excretion and tumor size in our study has important implications for basic research and diagnostic and therapeutic studies. If increased cyclic GMP excretion is observed with any types of tumors in patients, it could be useful for tumor diagnosis. The correlation of cyclic GMP excretion and tumor size and its alteration with irradiation, chemotherapy, or tumor removal also suggest that cyclic GMP excretion may be useful clinically in the assessment of therapeutic response and tumor recurrence in some patients. In any event, cyclic GMP excretion in rats bearing hepatomas can be used as an index of tumor growth and regression and should prove useful in examining this model system. Cyclic nucleotide excretion in animals and patients with other tumors and the source of the cyclic GMP in urine remain to be determined.

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Retinoic Acid Binding Protein: Occurrence in Human Tumors

Abstract. *Extracts of human carcinomas from lung and breast contain a protein which binds retinoic acid (vitamin A acid) with high specificity. The binding protein was not detected in normal lung or breast tissue from the same patients.*

The importance of vitamin A (retinol) for normal cellular differentiation of epithelial cells is firmly established (*1*). Vitamin A acid (retinoic acid) is produced in small quantities in vivo from retinol (*2*) and, if administered in the absence of retinol, can support growth and development of the rat but will not maintain vision (*3*) or reproductive function (*4*). The molecular mechanism of action of these compounds is obscure. Recently we have described the presence of two cellular binding proteins in rat tissue, one specific for retinol (*5*) and the other specific for retinoic acid (*6*). They are present in organs in very small quantities (0.3 to 1.5 μg per gram, wet weight) and possess high specificity for their respective ligands. We have found that the ability of *cis*-isomers of retinol to bind to the cellular retinol binding protein parallels their potency in promoting growth in the retinol-deficient rat (*7*), suggesting the involvement of the binding proteins in the mechanism of vitamin A action. Here we report the occurrence of the specific binding protein for retinoic acid in extracts from a limited number of human carcinomas. We could not detect the binding protein in extracts of nonmalignant tissue of the same organ from the same patients.

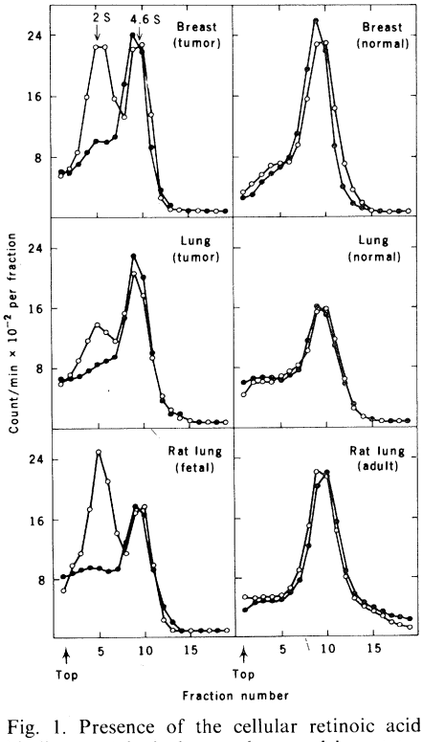

Fig. 1. Presence of the cellular retinoic acid binding protein in human lung and breast tumors and rat fetal lung. Portions of the tissue extracts were incubated with 40 nmole of [^{3}H]-retinoic acid (○) and with an additional 8 μmole of unlabeled retinoic acid (●). Portions (0.2 ml) of the incubation mixtures were subjected to sucrose gradient centrifugation for 20 hours at 148,000*g*.

Malignant and normal tissue were obtained from specimens removed surgically at Vanderbilt University Hospital. Routine diagnostic techniques of the surgical pathology laboratory were used to assign the designation malignant or normal to the tissue specimens (*8*). Fresh samples were stored at −20°C until assay. Extracts were prepared by homogenization of the tissue in two volumes of 0.05*M* tris(hydroxymethyl)aminomethane hydrochloride, *p*H 7.5, using a Polytron PT 10 homogenizer, followed by centrifugation at 31,000*g* for 10 minutes. The supernatant liquid was collected and titrated to *p*H 5 at 4°C with 1*M* acetic acid. The precipitated protein was removed by centrifugation at 31,000*g* for 10 minutes and the supernatant liquid (extract) retitrated to *p*H 7.5 with 1*M* NaOH.

The extracts were tested for the presence of a binding protein for retinoic acid by incubating portions with 40 nmole of all-*trans*-[11,12-^{3}H]retinoic acid (1.45 c/mmole) in the dark at 4°C for 4 to 5 hours. In order to determine the specific binding, duplicate portions containing 8 μmole of unlabeled retinoic acid were used. The ligands were added in 5 μl of ethanol containing 1 mg of α-tocopherol per milliliter. Immediately before sucrose gradient centrifugation, incubation mixtures were treated by adding 0.2 ml of a charcoal dextran solution (*9*) to absorb free retinoic acid. The charcoal-coated dextran was removed after a 5-minute incubation by centrifugation at 1000*g* for 5 minutes. A portion (0.2 ml)

of the clear supernatant was layered on a linear 5 to 20 percent sucrose gradient of 5 ml and centrifuged at 148,000*g* for 20 hours. The gradients were fractionated by piercing the bottom of the tube and collecting 0.25-ml fractions. Radioactivity in each fraction was determined by liquid scintillation counting, as described earlier (*5*).

From our previous work with rat tissue, the presence of the binding protein is indicated by a peak of radioactivity in the 2*S* region of the gradient, which is reduced when unlabeled retinoic acid has been added. A second peak at 4.6*S* is frequently observed and, in contrast to the 2*S* peak, this 4.6*S* binding is not diminished by excess unlabeled retinoic acid. It appears to be nonspecific binding to serum albumin which contaminates most preparations (*10*).

As shown in Fig. 1, a peak of radioactivity sedimenting in the 2*S* region of the gradient was observed for malignant tissue from human lung and breast. Normal tissue from the same organ did not show the specific binding protein at 2*S* but only the nonspecific binding at 4.6*S*. A total of four carcinomas have been examined (one lung and three breast) and all showed 2*S* binding indicative of the retinoic acid binding protein.

The human retinoic acid binding protein detected in these tumors is specific for retinoic acid as binding is diminished by excess all-*trans*-retinoic acid only, but not by retinol, retinal, or oleic acid. The molecular weight of this protein is approximately 15,000 daltons, when calculated from the sedimentation data by the method of Martin and Ames (*11*). Gradients with standard proteins were treated in parallel for calibration.

While we did not detect the retinoic acid binding protein in normal breast and lung tissue, we have observed its presence in the human uterus (*12*). This is similar to the situation in the rat, in that the lung of the adult rat shows no evidence for the binding protein but it is present in the uterus (*6*). However the cellular retinoic acid binding protein is clearly evident in rat fetal lung (Fig. 1) at day 19 of gestation. Currently it is not possible for us to extend our observations to human fetal material, but the presence of the retinoic acid binding protein in malignant tissue may be another case of the occurrence of a cellular component in malignant tissue which normally is observed only at the fetal stage, as has been reported for some other proteins (*13*).

There is considerable evidence, recently reviewed (*14*), that vitamin A status or administration of retinol, retinoic acid, or their derivatives affects the development of some tumors (*15*). Recently Bollag (*16*) showed that administration of an aromatic analog of retinoic acid leads to a marked regression of carcinogen-induced established skin papillomas and carcinomas of mice. However, the compound has no significant effect on transplantable tumors examined, including Ehrlich ascites carcinoma. It is of interest that we find no detectable retinoic acid binding protein in this carcinoma (*17*). The presence of the binding protein may be required in order for such an antimetabolite of retinoic acid to have a therapeutic effect.

The observations reported here were made with a limited number of human carcinomas which all contained a high proportion of epithelial cells. Since in the samples of normal tissue there was also a considerable amount of these cells (*8*), the presence of the retinoic acid binding protein represents a difference between malignant and normal cells rather than a drastic change in epithelial content. The occurrence of a cellular retinoic acid binding protein indicates that at least in some malignant tissues there is an altered interaction of retinoic acid within the cell compared to normal tissue.

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8. The breast carcinomas were all of the infiltrating duct type with demonstrated metastasis to axillary lymph nodes in each case. The lung carcinoma was of the nonkeratinizing epidermoid type. Histologic examination of each analyzed sample demonstrated a great amount of interstitial collagen formation in one of the breast carcinomas and a normal component of epithelial elements in each of the samples of normal tissue. Special effort was made to exclude fat from the breast samples and bronchial elements from the normal lung samples, although the lung sample contained bronchiolar elements and normal alveolar elements. Histologic examination of the relative numbers of epithelial cells indicated approximately 75 percent in the lung tumor, whereas the normal lung tissue contained 50 percent epithelial cells. Breast tumors ranged from 80 to 95 percent epithelial cells while normal samples were 50 to 85 percent.
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Differential Fluorescent Staining of Human Chromosomes with Daunomycin and Adriamycin—The D-Bands

Abstract. *Human chromosome preparations were treated with a group of anthracycline antibiotics. Well-defined, orange-red fluorescent bands were observed on chromosomes after the slide was stained with daunomycin and adriamycin but not with nogalamycin. The characteristic differential bands appeared to be similar to the banding patterns obtained by the quinacrine techniques. Interaction of these antibiotics with DNA could provide information on the general mechanism of fluorescent banding. Further, these bands (D-bands) appeared to be more stable than the Q-bands and may have some usefulness for routine clinical cytogenetic analysis.*

Fluorescent labeling of chromosomes by quinacrine and quinacrine mustard (Fig. 1a) and other DNA-binding fluorochromes has become a well-established technique for the identification and analysis of chromosomes and chromosome regions (*1*). Intercalation is thought to be the primary mode of binding of the fluorochromes to chromosomal DNA (*2*). In the interaction of quinacrine with chromosomes, the banding patterns appear to be derived from the variable quantum efficiency of quinacrine fluorescence as a function of the base composition around the intercalation site (*3*). Recently, it was shown that uninterrupted stretches of A-T (adenine-thymine) base pairs enhance quinacrine fluorescence, while a progressive quenching of quinacrine fluorescence is observed with increasing guanine content of the DNA (*4*). It has also been shown that several factors which affect the quenching or enhancement of fluorescence by native DNA with specific nucleotide sequences corresponds well with the effect of brightness and differentiation of Q-bands on the