Histological Observations of
Hepatocellular Carcinomas of Rats Fed Varying Amounts of Vitamin A[1]

Dorothy M. Morre, Paula C. Fink[2], Thomas Kloppel[2] and Rachel Brody[3]
Department of Foods and Nutrition, School of Consumer and
Family Sciences, Purdue University, West Lafayette, Indiana 47907

Introduction

Vitamin A is known to be required in the normal pathway of epithelial cell differentiation in essentially all of the epithelial target sites of origin of cancer (4, 9). Carciongenesis involves a de-differentiation or at least an abberant differentiation in these epithelia. Vitamin A used in pharmacological amounts in cancer prevention is an attempt to arrest or reverse a pathological process by enhancement of the physiological processes of differentiation (8).

Many investigators have postulated that the phosphate ester of retinol, retinyl phosphate, may function in the glycosylation of glycoproteins (See 6 and 10 for literature). While it is premature to accept a metabolic function of vitamin A in connection with the transfer of mannose, galactose or other sugars to glycoprotein, it would be of interest if such a reaction were one function of the vitamin. This connection might be facilitated by a better understanding of the cellular basis of vitamin A effects. In this study, we evaluated histochemically the effects of vitamin A on glycoprotein synthesis in liver and tumor tissue with the aid of periodic acid-Schiff (PAS) reagent.

Materials and Methods

Weanling male rats of the Charles River strain were randomly divided into 3 groups of 10 each and fed diets containing no vitamin A, adequate amounts or excessive amounts of vitamin A (100X adequate) (Table I). Vitamin A was in the form of retinyl acetate. Food intake and weight gain were recorded weekly. Rats were fed respective diets for two weeks prior to being subcutaneously injected with transplantable hepatocellular carcinomas of the rat derived from solid tumors induced by N-2-fluorenylacetamide. Animals were examined daily for tumors. Tumor mass was estimated from weekly measurements with a vernier caliper of two tumor dimensions. The following mathematical formula was used to determine approximate tumor mass: $M = 4/3 \pi (a^2/4)b/2$ where a and b are the two dimensions (perpendicular axes) measured and M = mass (7).

Vitamin A concentration of liver and tumor mass was determined using the Bayfield (1) procedure. Protein concentration was determined for liver and

TABLE I *Composition of basal diet used in tumor growth studies*

Ingredient	Percent
Casein	20.0
DL-Methionine	0.3
Cornstarch	15.0
Sucrose	50.0
Fiber (Cellulose Type)	5.0
Corn Oil	5.0
Mineral Mix	3.5
Vitamin Mix	1.0
Choline Bitartrate	0.2

tumor mass by the Bradford (2) Coumassie Blue dye binding assay. For histological analysis, portions of the tumor tissue were fixed in 10% neutral buffered formalin, embedded in Paraplast and reacted with PAS reagent and counterstained with hematoxylin. Mitotic index was determined by using an ocular grid in a microscope and counting all of the cells which fell within the grid spaces. Then cells undergoing mitosis were counted in the same field. Mitotic index equals number of mitotic cells observed divided by the total number of cells.

Results

Tumors were fewer and appeared later under conditions of either vitamin A deficiency or excess. By day 21 post-injection, 10% of the animals in the group fed adequate amounts of vitamin A had tumors and by day 28, 90% of the animals in this group exhibited tumor growth, whereas in both the deficient and excess vitamin A groups there were no signs of tumors at day 21 with only 10% of the animals in both groups having tumors at day 28. Diets containing excess amounts of vitamin A thereafter continued to prevent tumor growth to a greater extent than did the diets containing no vitamin A. On day 42, 50% of the animals fed the vitamin A-free diets and 40% of the animals fed the excess vitamin A diets exhibited signs of tumor growth. However, two of the animals fed excess vitamin A diets had tumors that were too small to measure with the vernier calipers. The rats that did not have tumors by day 42 were allowed to continue on their respective dietary regimen for approximately another 100 days and did now show signs of tumor growth during the duration of the experiment. Liver vitamin A approximately paralleled intake while that in tumors reflected but did not parallel the amounts fed.

Mitotic indexes (Table II) were highest for the adequate group, lower for the vitamin A deficient group and lowest for the excess vitamin A group. Tumor

TABLE II *Mitotic indexes of hepatocellular carcinomas of rats fed varying amounts of vitamin A*

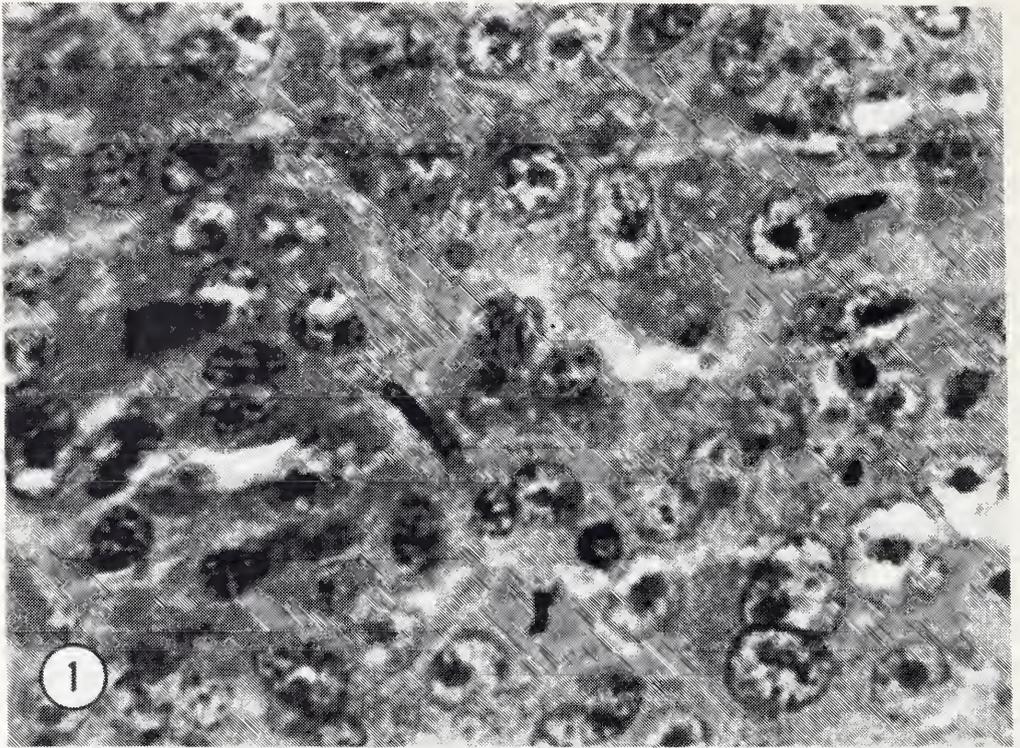| Dietary Vitamin A Treatment Group	Mitotic Index
Deficient	0.055 ± 0.003*
Adequate	0.085 ± 0.018
Excess	0.045 ± 0.010

*Mean ± SD

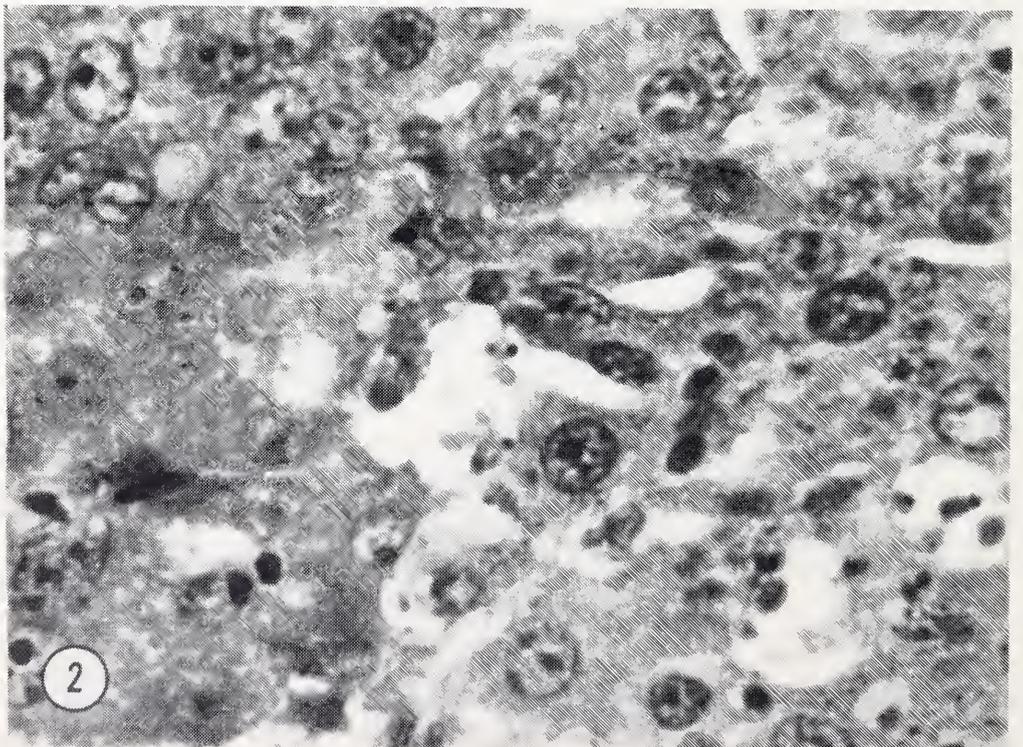Figure 1. *Hepatocellular carcinoma of a rat fed an excessive amount of vitamin A. Mag. = 800X.*

Figure 2. *Hepatocellular carcinoma of a rat fed an adequate amount of vitamin A. Mag. = 800X.*
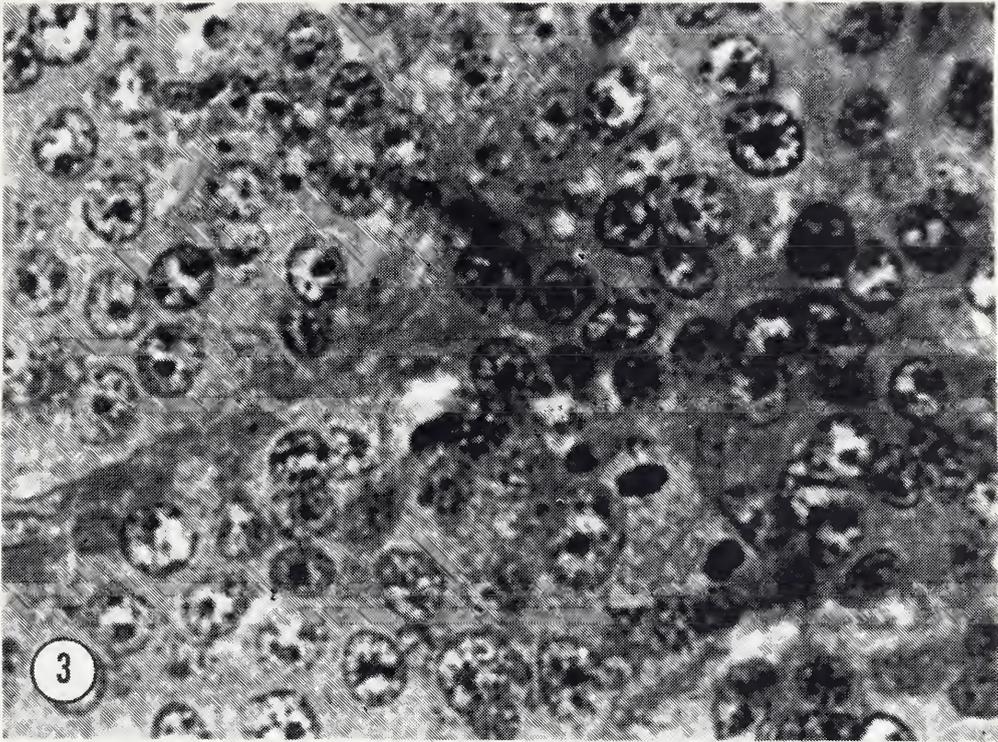
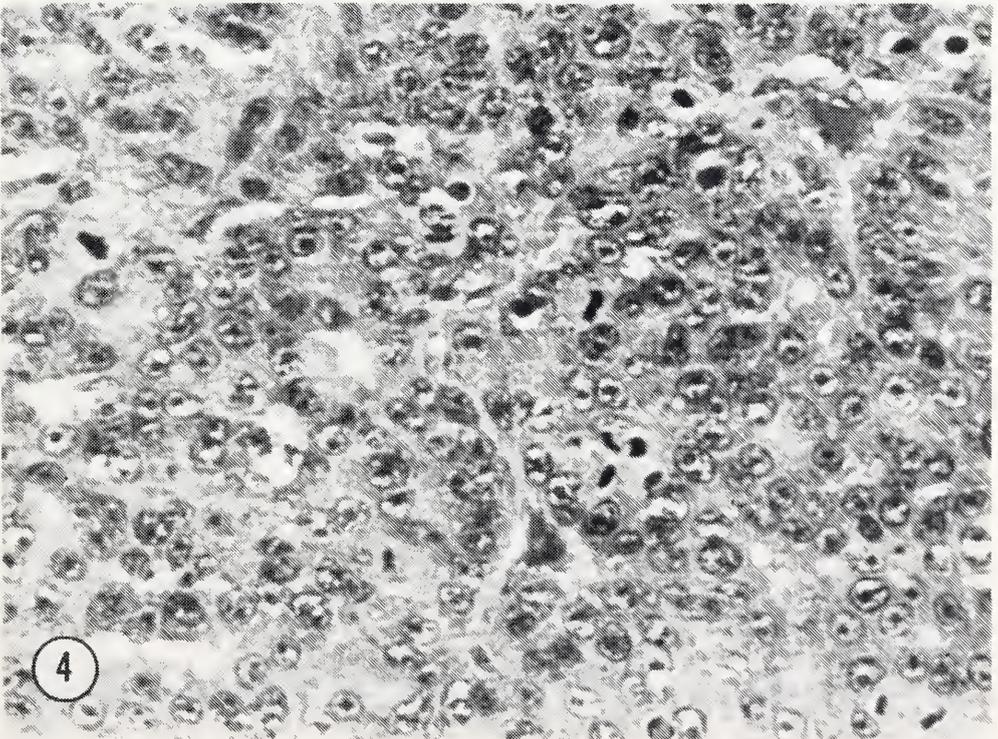FIGURE 3. *Hepatocellular carcinoma of a rat fed a deficient amount of vitamin A. Mag. = 800X.*

FIGURE 4. *Low magnification photomicrograph of a hepatocellular carcinoma of a rat fed a deficient amount of vitamin A. Mag. = 400X.*

tissue in the adequate group (Fig. 1) appeared less consistent in cell size with increased number of large cells with very large and pleomorphic nuclei than did those in both the excess and deficient groups (Figs. 2 and 3). Nuclei were smaller and more uniform in diameter with both dietary extremes that in the group fed adequate vitamin A. More vacualor spaces were observed in the excess group compared to the adequate. This pattern of cavitation was reminiscent of normal sinusoid patterns and was observed in both the excess (Fig. 2) and the deficient animals (Fig. 3 and 4). PAS reactivity of cell borders and intracellular spaces appeared to be greater in the hepatomas of the excess and deficient groups compared to the adequate group as did the overall level of cytoplasmic staining.

Discussion

Very few nutritional investigations have been directed toward the prevention of cancer. Vitamin A, used as an agent to prevent epithelial cancer, is attracting increasing attention. Moon et al. (4) reported reduced incidence of mammary cancer as well as a marked reduction in the total number of cancers and benign tumors when vitamin A in the form of retinyl acetate was fed to rats following injection with the carcinogen, N-methyl-N-nitrosourea. Our findings show that either a lack of vitamin A or excessive amounts of vitamin A in the diet prevent growth of subcutaneously injected hepatocellular carcinomas. An equivalence of dietary vitamin deficiency and excess in chemoprevention is surprising but has been observed previously in studies of lysosome lability (3). The mechanism involved in prevention of tumor growth is not known.

Mitotic indexes were highest for the adequate group with those for the vitamin deficient and excess groups being the lowest which suggests that cell division was slowed by the dietary extremes. Most interesting, the pattern of tissue organization of the hepatomas of the animals fed either vitamin A excess or deficient diets was very different from that of the original poorly-differentiated hepatomas transplanted. This poorly-differentiated morphology with attendant loss of tissue organization persisted throughout the study in those animals fed diets containing adequate amounts of vitamin A. However, in the dietary extremes, in those showing tumor establishment and growth a pattern of cavitation was observed reminiscent of normal sinusoid patterns. These observations suggest strongly a return to a more normal, liver-like stage of differentiation in the few tumors that persisted in the two animal groups representing dietary extremes.

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