

Autoradiographic Localization of Vitamin A in the Kidney of Rats¹ (39758)C. G. PLOPPER, D. L. WALLACE, T. J. BUCCI,² AND H. E. SAUBERLICH*Letterman Army Institute of Research, Presidio of San Francisco, California 94129*

Previous work has established that the kidney has a functional role in vitamin A metabolism and that it is the second most important storage site for vitamin A (1). When weanling rats were given a vitamin A-free diet, their tissues became depleted of the vitamin in the sequence of liver, blood, and kidney (2, 3). In the rat, the kidney has been suggested as a site of formation of retinoic acid from retinol (4). In addition, catabolism of plasma retinol-binding protein (RBP) occurs primarily in cells of proximal renal tubules (5). Peterson *et al.* (6) have postulated that a large portion of the vitamin is delivered to the cells by glomerular filtration and tubular resorption.

The purpose of this study was to define autoradiographically the distribution of vitamin A in the kidney and to support the concept of a role for the kidney in vitamin A metabolism.

Materials and methods. Weanling male rats (Holtzman) were fed *ad libitum* the following semisynthetic vitamin A-deficient diet: cornstarch-cerelose (1:1), 66.8%; vitamin-free casein (ICN Nutritional Biochemicals, Cleveland, Ohio), 22.0%; corn oil, 5.0%; Bernhardt-Tomarelli salt mix, 4.0%; DL-methionine, 0.2%; water-soluble vitamin mix, 2.0%. The water-soluble vitamin mix supplied the following concentrations (mg/100 g of diet): thiamin-HCl,

0.28; riboflavin, 0.56; pyridoxine-HCl, 1.56; vitamin B₁₂, 0.0011; niacin, 3.34; Ca-D-pantothenate, 1.78; folic acid, 0.20; biotin, 0.06; and choline chloride, 166.66. The fat-soluble vitamins were administered orally three times weekly in the following quantities per 0.05 ml of corn oil: vitamin D₃, 1.56 μ g; α -tocopherol acetate, 2.62 mg; and menadione, 3.36 μ g.

In about 6 weeks, after body weights had plateaued and a 10% weight loss had occurred, three vitamin A-deficient rats were anesthetized with Penthrane³ and their jugular veins were exposed. Tritiated vitamin A as [11, 12-³H]retinyl acetate (0.363 mCi/animal, sp act 253 μ Ci/mg; kindly donated by Hoffman LaRoche, Switzerland) was administered intravenously. The carrier solvent was ethanol:Tween 80:1% bovine serum albumin in physiological saline (1:1:16). Three hours after label injection, the animals were reanesthetized and their abdominal cavities were opened. Retrograde perfusion was performed through the abdominal aorta with heparinized saline, followed by fixation of the tissue by perfusion with 300-500 ml of Karnovsky's fixative diluted 1:1 with distilled water (pH 7.2) (7). The fixed kidneys were removed and entire cross sections, 0.5 to 1 mm in thickness, were cut with razor blades. The sliced tissue was processed by a rapid embedding technique developed in this laboratory which preserved lipid-soluble substances. The procedure permitted over 98% retention of radioactive label (8). The slices were immersed in osmium tetroxide, dehydrated in 75 and 95% ethanol and embedded in Epon/araldite (8). Epoxy sections, 1- μ m thick, were cut with glass knives on a JB-4 microtome⁴ and coated with NTB-2⁵ autoradiographic emulsion, diluted 1:1 with distilled water. Five to seven weeks later, the

¹ In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal Resources, National Academy of Sciences-National Research Council.

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⁵ Eastman Kodak, Rochester, N. Y.

autoradiographs were developed in Vividol⁶ and stained with methylene blue-azure II and basic fuchsin for light microscopy.

Results. A minimum of six slides was examined by light microscopy for each tissue slice. Grain distribution was similar in all sections examined. Reduced silver grains, indicating the presence of labeled vitamin A, were observed over three types of cells in the rat kidney: lining cells in proximal convoluted tubules, capillary endothelium in the medulla, and unidentified cells in the cortical interstitium. Grains were present over all the proximal convoluted tubules of the kidney cortex (Figs. 1A and B). The label was diffusely distributed over the cells and was observed in equal density over the microvilli and the cytoplasmic contents, but not the nucleus. The density varied among individual tubules, but was always greater than background and that over glomeruli (Fig. 1A), distal convoluted tubules (Fig. 1B), and collecting ducts. Dense grain accumulations, primarily over the cytoplasm, were present in a small number of cells in the interstitium of the cortical region (Fig. 1C). These cells were either rounded, oblong, or spindle-shaped and had central nuclei. Endothelial cells of capillaries in the medulla contained on occasion heavy label (Fig. 1D), but no specific distribution of labeled endothelial cells was observed. Glomeruli, distal convoluted tubules, collecting ducts, and thick and thin loops of Henle were without remarkable grain accumulations.

Discussion. Goodman *et al.* (1) showed that 93% of the radioactivity recovered from the kidney 3 hr after injection of labeled retinyl acetate was associated with vitamin A. The tissues in the current study were collected after the same interval and the located radioactivity has been assumed to represent vitamin A. In other unpublished studies, after administration of labeled retinyl acetate, more than 90% of the radioactivity present in the kidney was located in retinyl esters with the remainder located largely in retinol. Similar results were reported by Ito *et al.* (9).

The kidney has been shown to participate in the conversion of retinol to retinoic acid

and to have the highest concentration of vitamin A 3 hr after intravenous administration of labeled retinyl acetate to deficient rats (4). In the current study, three separate cell types contained radioactivity: proximal convoluted tubule epithelium, capillary endothelium, and interstitial cells. The majority of the label was present in tubular epithelium. While density of label was greater over occasional endothelial and interstitial cells than over individual tubule cells, the latter were vastly more numerous and accounted for most of the label. Therefore the proximal convoluted tubule is probably quantitatively the most important renal site of retinoic acid formation.

The present observations are supported by previously reported work in which vitamin A was localized by fluorescence microscopy (10). In that study with normal rats, vitamin A was observed in the endothelium of capillaries surrounding a small percentage of the nephrons. However, vitamin A was not seen in the kidneys of vitamin A-deficient animals. The vitamin was found in proximal convoluted tubules only in animals given large doses of vitamin A. This led the authors to conclude that the role of the kidney was in excretion of vitamin A. Results of the present study, using a more sensitive technique, would indicate that vitamin A is in the proximal convoluted tubules and capillaries of vitamin A-depleted rats 3 hr after intravenous administration of radioactive vitamin A. Even though the administered dose was unphysiologically large, the results suggest that the kidney repletes quickly and preferentially.

Some evidence of renal metabolism of retinol-binding protein (RBP), as distinct from vitamin A, is available (5). Smith *et al.* (11) have proposed that renal tubules resorb and metabolize RBP. Peterson *et al.* (6) found radioactive vitamin A in isolated cells from the proximal convoluted tubules and proposed that the kidney salvages filtered retinol. The present study provides additional evidence that proximal tubules are the major renal site of vitamin A localization. The specific role of the kidney in the metabolism of vitamin A remains uncertain, however.

Vitamin A appears to be filtered by the glomerulus and resorbed by tubular epithe-

⁶ GAF Corp., New York, N.Y.

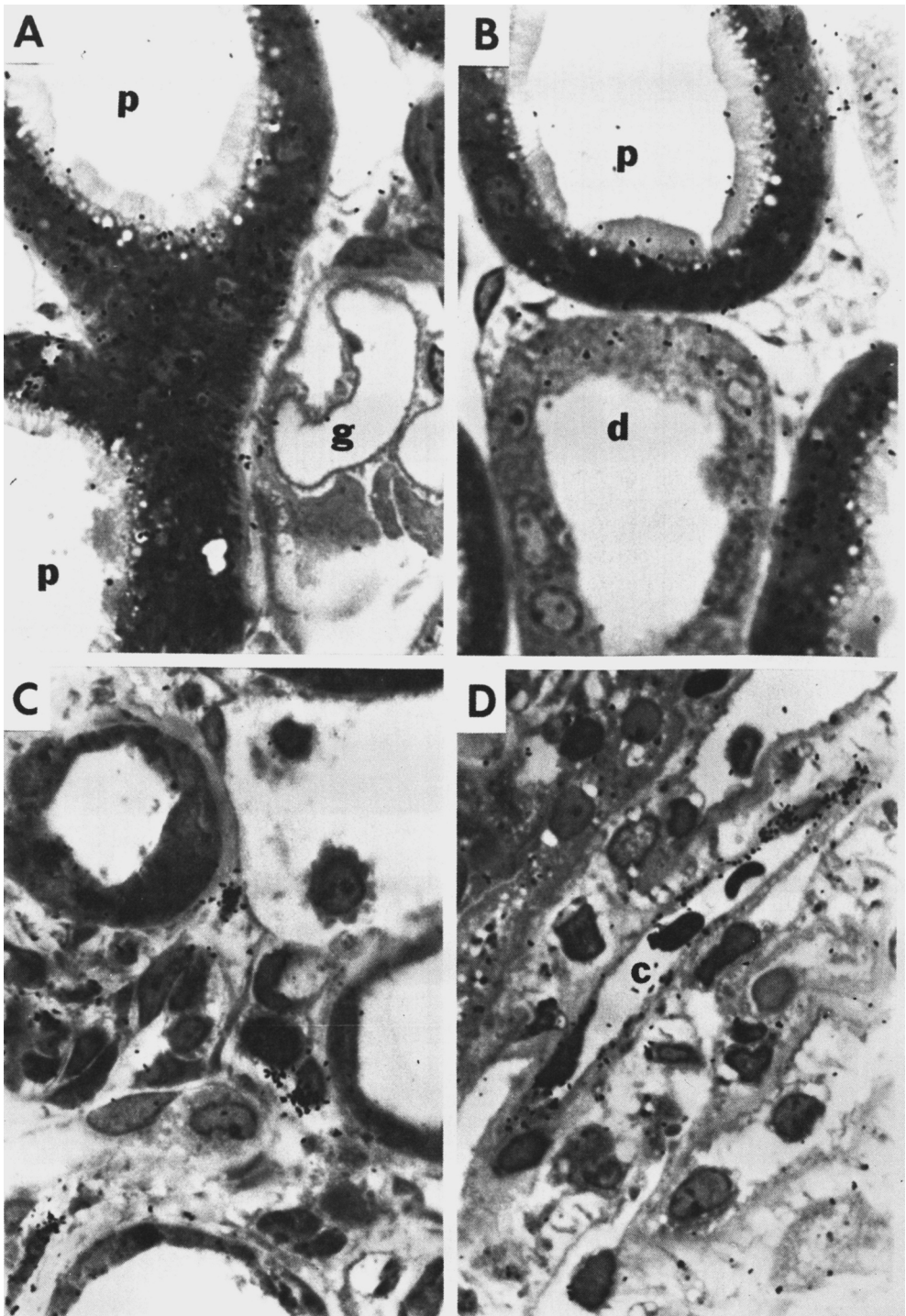


FIG. 1. Light microscopic autoradiographs of kidney from vitamin A-deficient rats given $[11, 12\text{-}^3\text{H}]$ retinyl acetate (0.363 mCi/animal) intravenously 3 hr prior to killing (stained with methylene blue-azure II and basic fuchsin). (A) Proximal convoluted tubules (p) and glomerulus (g) in cortex. $2000\times$. (B) Proximal (p) and distal (d) convoluted tubules in cortex. $2000\times$. (C) Labeled cells in interstitium of cortex. $2240\times$. (D) Labeled capillary (c) in medulla. $1840\times$.

lium which may permit the recirculation and/or conversion of retinol to retinoic acid. The presence of vitamin A in certain interstitial and endothelial cells is unexplained.

Summary. The distribution of vitamin A in the kidney was investigated autoradiographically. Three hours after intravenous administration of [11, 12-³H]retinyl acetate to vitamin A-deficient rats, the vast majority of the label was present in cells of the proximal convoluted tubules. A very small number of endothelial cells in the medulla and interstitial cells in the cortex were also labeled. The evidence strongly suggests that the proximal convoluted tubule is the major renal site for vitamin A localization. The precise role the kidney plays in overall vitamin A metabolism remains uncertain.

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