Storage of vitamin A in extrahepatic stellate cells in normal rats

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Abstract In mammals, vitamin A is primarily stored as retinyl esters in hepatic stellate cells under normal dietary intake of the vitamin. Previously, extrahepatic vitamin A-storing stellate cells have only been identified in animals maintained on a vitamin A-rich diet, and it has not been known whether these cells play a role in normal vitamin A metabolism. The purpose of this study was, to quantify the stellate cell lipid droplet area in hepatic and extrahepatic stellate cells in control rats and in rats fed excess vitamin A. The stellate cells were identified by the gold chloride staining technique, specific autofluorescence of retinyl ester, and by electron microscopy. The stellate cell lipid droplet area was then quantitated by the use of morphometric quantitation. We demonstrated that lipid droplet-containing stellate cells were identified in liver, lung, kidney, and intestine, in normal as well as vitamin A-fed rats. The area of lipid droplets in liver, lung, and intestine stellate cells of normal rats was 0.2, 0.3, and 0.04 mm^2 per cm^2 tissue, respectively. When the rats were administered excess vitamin A, the hepatic, lung, and intestinal stellate cell lipid droplet area increased about 10-fold, 2-fold, and 40-fold, respectively. Thus the present study shows that extrahepatic stellate cells in lung and intestine of normal rats contain lipid droplets, and that these lipid droplets increase in area when high doses of vitamin A are fed to the animals. These data suggest that not only liver stellate cells but also extrahepatic stellate cells play an important role in vitamin A storage in normal as well as vitamin A-fed animals.—**Nagy, N. E., K. B. Holven, N. Roos, H. Senoo, N. Kojima, K. R. Norum, and R. Blomhoff.** Storage of vitamin A in extrahepatic stellate cells in normal rats. *J. Lipid Res.* 1997. **38:** 645–658.

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In mammals, between 50 and 80% of the total amount of vitamin A (retinoids) is stored in liver. This liver store is almost exclusively localized to the hepatic stellate cells (also called fat-storing cells, Ito cells, and lipocytes) (1). The hepatic stellate cells are localized within the space of Disse, i.e., the interstitial space between the parenchymal and the endothelial cells. The stellate cells account for only approximately 7% of the number of liver cells (2).

A characteristic ultrastructural feature of the hepatic stellate cell is lipid droplets in the cytoplasm (3, 4). The lipid droplets of rat hepatic stellate cells consist of about 40% retinoids, 30% triacylglycerol, and 15% cholesteryl ester. The composition of the lipid droplets is dependent on the dietary retinoid intake. In rats fed a high retinoid diet (10 times the normal level), the relative content of retinoids increased to about 65% and the triglyceride and the cholesteryl ester content decreased to about 24% and 7%, respectively (5). The composition of the lipid droplets in rats fed a high triglyceride diet was similar to that of the lipid droplets in rats fed a normal diet.

Small amounts of retinyl esters, the storage form of retinoids, have also been found in tissues such as lung, kidneys, and intestine. Morphological studies using vitamin A-fed animals or animals that consume high amounts in their normal diet (such as lamprey and some polar animals) have suggested that the extrahepatic storage of retinoids is localized to a cell type with a similar phenotype as the hepatic stellate cell (3, 4, 6, 7). These extrahepatic stellate cells also often contain lipid droplets, they have characteristic cytoplasmic projections, and they are localized interstitially (3, 4, 8). We have applied the same definition of extrahepatic stellate cells in our study. None of the previous studies have,

Abbreviations: CRBP, cellular retinol-binding protein; FITC, fluorescein isothiocyanate; HPLC, high performance liquid chromatography; RBP, retinol-binding protein; TEM, transmission electron microscopy; TMMP-retinol, all-*trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraen-1-ol.

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however, demonstrated that extrahepatic stellate cells stored retinoids when the animals consumed a diet with a vitamin A content that is considered normal for most animals.

To study whether extrahepatic stellate cells in rats fed a normal diet store retinoids, we have examined sections from lung, kidney, and intestine of these animals by two morphological methods specific for vitamin A. First, we used the gold chloride staining technique which has been demonstrated to yield specific precipitations in lipid droplets containing retinyl esters (9). Second, sections were analyzed for specific autofluorescence of retinyl esters (exitation at 328 nm, emission at 480 nm). Neither of these techniques gave positive results with regard to storage of retinoids in extrahepatic stellate cells of normal rats, probably due to low sensitivity of these methods. However, when we quantitated the total lipid area in extrahepatic tissues from rats fed a normal diet or a high vitamin A diet, we found that the areas of lipid droplets in liver, lung, and intestine stellate cells of normal rats were 0.2, 0.3, and 0.04 mm^2 per cm^2 tissue, respectively. In the vitamin A-fed rats, these values increased to 2.2, 0.7, and 1.5 mm^3 per cm^2 tissue, respectively.

Thus, the present study shows that extrahepatic stellate cells in lung and intestine of normal rats contain lipid droplets, and that these lipid droplets increase in volume when high doses of vitamin A are fed to the animals.

EXPERIMENTAL PROCEDURES

Animals and diets

Nine normal male Wistar rats, weighing 294 g ± 10.6 g, were fed different diets of vitamin A during an experimental period of 18 days. The control group (n = 3) was maintained on an ordinary pelleted diet (Type R3, Ewos AB, Södertälje, Sweden), consisting of 22% protein, 5% fat, and 51% carbohydrates (w/v). The vitamin A group (n = 3), in addition to the 12.6 µmol retinol/kg supplied by the ordinary pellet, was fed an oral dose of 48.9 µmol retinol as retinyl ester in 2 ml groundnut oil (Afi A Diagnosticum, Nycomed, Oslo, Norway) every second day. During the experimental period the vitamin A group received a total of 471.4 µmol retinol. The groundnut oil group (n = 3) was fed the same volume of pure groundnut oil as the vitamin A group, and received vitamin A only from the ordinary pellet. The oil solutions were given by tube feeding. Before tube feeding, the rats were fasted 12 h. At the end of the treatment period, the rats were killed 3 days after the last intake of vitamin A/groundnut oil, and the rats were fasted overnight before being killed.

The growth rates of the rats were assessed during the experimental period. The growth rate was somewhat faster in the control group (weight at the end of experimental period; 319 g ± 4.5 g) than in the vitamin A group (weight at the end of experimental period; 309 g ± 4.5 g), and the groundnut oil group (weight at the end of experimental period; 302 g ± 3.7 g). The rats in the vitamin A group showed, however, no clinical signs of vitamin A toxicity.

Chemicals

TMMP-retinol (RO 12-0586) was kindly donated by Hoffman La Roche (Basel, Switzerland). Trizmabase and pyrogallol were obtained from Sigma Chemical Co. (St. Louis, MO), and gold chloride was obtained from Janssen Chimica (Beerse, Belgia). Sodium cacodylate, osmiumtetroxid, Spurr resin, lead citrate, and uranyl acetate were purchased from TAAB Laboratories (Equipment Ltd., UK), and glutaraldehyde (50%) was purchased from Electron Microscopy Sciences (Port Washington, PA). Rabbit anti-chicken gizzard desmin monoclonal antibody (PS 31) was purchased from Sanbio BV/Monosan (Am Uden, Netherlands) Mouse anti NH2 terminal synthetic decapeptide of α-smooth muscle actin monoclonal antibody (A 2547) and FITC-labeled goat anti-mouse IgG (F 6897) were purchased from Sigma. FITC-labeled goat anti-rabbit IgG (65-6111) was obtained from Zymed Laboratories (San Francisco, CA). Ethanol was delivered by A/S Vinmonopolet (Oslo, Norway). All other chemicals of analytical grade were obtained from Merck (Darmstadt, Germany).

Retinol analysis

Total retinol (refers to the sum of the concentration of both free retinol and retinyl esters) in liver, kidney, lung, and intestine was determined in three animals from each diet group by HPLC. For quantitation, TMMP-retinol (all-*trans*-9-(4-methoxy-2,3,6-trimethylphenyl)-3,7-dimethyl-2,4,6,8-nonatetraen-1-ol) was used as an internal standard (10). After the rats were killed, 1–3 g of tissues was removed and homogenized in Tris-buffer (0.25 M, pH 7.8). The homogenates (2–10%) were hydrolyzed with potassium hydroxide (10%) in ethanol with pyrogallol (1%), and extraction into hexane was performed as described by Blomhoff et al. (11). The samples were injected automatically on the HPLC system that included a model 590 pump from Waters associates (Milford, MA), and a detector monitoring at 326 nm. The separation was effected on a 5-µm Supel-

cosil RP-8 column, 25 cm × 4.6 mm (Supelco, Inc., Bellafonte, PA), with methanol–water (95:5 (v/v) as a mobile phase at a flow rate of 1.5 ml/min.

Analysis of triacylglycerol

The triacylglycerol concentration in the different tissue homogenates (liver, lung, kidney, and intestine) from three animals in each diet group was determined by an enzymatic colorimetric test from Boehringer Mannheim Biochemica (Mannheim, Germany). The samples were measured spectrophotometrically on a Technicon RA 1000 (Technicon Instruments Corporation, Tarrytown, NY).

Vitamin A detection by the gold chloride method

The gold chloride staining method is used to visualize vitamin A by light microscopy. Vitamin A precipitates with gold on the surface of lipid droplets containing the vitamin (9). The method used was a modified version of the original von Kupffer method (7, 9). Tissues for staining were cut into blocks 10–20 × 10–15 × 5 mm, and fixed in formaldehyde (3.7%) for 0.5–2 h at room temperature. Sections (20–50 μm) were cut on a freezing microtome and subsequently incubated for 8 h with 0.01% gold chloride solution containing 0.01% hydrogen chloride (pH 2.8), in darkness at 22°C. Dehydration was done with graded ethanol (70%, 90%, 96%, and 100%) and finally xylene. The sections were then mounted, dried, and examined in a Leitz light microscope.

Vitamin A detection by fluorescence microscopy

To detect vitamin A, frozen sections from the different organs were examined in a fluorescence microscope as described previously (9, 12). The formaldehyde (3.7%)-fixed sections (20–50 μm) were mounted in distilled water and immediately observed under the fluorescence microscope (Leitz light microscope equipped with a fluorescence luminescence unit). When excited by 326 nm ultraviolet light, vitamin A emits a natural, green fluorescence that characteristically fades away within 10 to 20 sec.

Immunocytochemical staining of desmin and α-smooth muscle actin

Male Wistar rats maintained on a normal diet were fasted for 18 h before being killed. The animals were anesthetized and subsequently perfused intracardially with 0.1 M phosphate-buffered saline (PBS) (pH 7.4), followed by fixation with 4% paraformaldehyde (0.2% picric acid in 0.1 M PBS, pH 7.4). Sections (5 mm × 5 mm) obtained from the liver, kidney, lungs, and intestine were postfixated in the same buffer at 4°C for 18 h. Cryostat sections (5–8 μm in thickness) were mounted on siliconated non-fluorescence objective glasses and air-dried for 2 h at room temperature. The sections were rinsed three times in 10 mM PBS for 5 min, and blocked for nonspecific binding of antibody by incubation with 1% bovine serum albumin (BSA) in PBS for 30 min at room temperature. For immunofluorescence of desmin, the sections were first incubated with rabbit anti-desmin monoclonal antibody (1:100 in 2% normal goat serum, and 1% BSA in PBS) at 4°C for 18 h. The sections were subsequently rinsed three times with PBS for 5 min, and finally incubated with FITC-labeled goat anti-rabbit IgG (1:100 in 1% BSA/0.1% Triton in PBS) for 1 h, rinsed 3 times with PBS for 5 min, mounted in glycerin/TBS (0.15 M NaCl in 50 mM Tris/HCl, pH 7.5), and observed under confocal laser scanning microscope (Zeiss, LSM 410). For immunofluorescence of α-smooth muscle actin, the sections were first incubated with mouse anti-α-smooth muscle actin monoclonal antibody (1:100 in 2% normal goat serum, and 1% BSA in PBS) at 4°C for 18 h. The sections were then rinsed 3 times with PBS for 5 min, and incubated with FITC-labeled goat anti-mouse IgG (1:100 in 1% BSA/0.1% Triton in PBS) for 1 h. Finally, the sections were rinsed 3 times with PBS for 5 min, and the sections were mounted in glycerin/TBS and observed under confocal laser scanning microscope (Zeiss, LSM 410).

Electron microscopy

Liver, lung, kidney, and small intestine were examined by electron microscopy for the accumulation of lipid droplets. Three rats in each group were anesthetized with pentobarbital (Norsk Medisinaldepot, Oslo, Norway) before the tissues were fixed by total body perfusion (13) with 2% glutaraldehyde in 0.1 M cacodylic acid (ph 7.4). Ultimately, small pieces of the organs were removed, put in the fixation solution, and immediately cut into small blocks of about 1 mm^3. After glutaraldehyde fixation for 24 h at 4°C, the fixation buffer was replaced by 0.1 M cacodylic acid buffer (pH 7.4) and the tissue pieces were washed twice for 10 min at 4°C. The samples were postfixed with 2% OsO_4 in 0.1 M cacodylic acid buffer (pH 7.4) for 2 h at 4°C and washed twice for ten min with the same buffer without OsO_4. After rinsing, the samples were dehydrated in an increasing gradient of ethanol (70%, 90%, 96%, and 100%) and embedded in Spurr resin. After polymerization of the resin at 70°C for 24 h, 50-nm sections were cut on an LKB III microtome using diamond knives. The sections were placed on formvar/carbon-coated copper slot grids (1 mm) and stained for 2 min with saturated uranyl acetate solution in 50% ethanol, and

TABLE 1. Concentration of total retinol in different tissues

Diet Group	Liver	Lung	Kidney	Intestine
	nmol/g tissue wet weight			
Control	1106 ± 131	19 ± 8	9 ± 2	2 ± 0.3
Vitamin A	16877 ± 1651***	966 ± 449**	224 ± 127***	711 ± 376*
Groundnut oil	967 ± 210	12 ± 2	4 ± 0.6*	2 ± 0.2

Total retinol = retinol plus retinylester was measured as described in Materials and Methods. The results presented are means ± SE; n = 3 in each diet group.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. control rats.

2 min in 0.2% lead citrate solution in 0.1 M NaOH. The sections were examined using a Jeol B 1200 transmission electron microscope and an accelerating voltage of 80 kV.

Morphometric analysis of lipid using transmission electron microscopy

In order to determine the effect of vitamin A administration on the lipid content in hepatic and extrahepatic stellate cells, we quantitated the area of the lipid droplets in the cells and related it to the total area of the tissue. The analysis was performed on three animals in each diet group. From each animal, four organs were analyzed, and from each organ, five sections were analyzed. Five micrographs (magnification of 6000×) were studied per section (i.e., 10 mm in the micrograph represents 1.67 μm). The total tissue area in a micrograph was calculated from the known magnification. The lipid droplet areas were estimated using a square point lattice test system (14, 15) consisting of 1200 points each representing 0.78 μm^2. The lipid areas were calculated by multiplying this factor by the number of points covering the lipid droplets. From the liver and lung, the sections were made randomly; in the intestine, however, the sections were taken systematically from the lamina propria. Due to the heterogenous localization and the rare appearance of the stellate cells in the kidneys, a morphometric quantitation was not performed.

Statistical analysis

All results are presented as means ± standard error of the mean (SE). Student's *t*-test was used for calculation of statistic significance of differences between group means.

RESULTS AND DISCUSSION

Tissue levels of total retinol

The content of total retinol in liver, lung, kidney, and intestine from the control, the vitamin A-fed, and the groundnut oil-fed groups is presented in **Table 1.** For all three dietary groups the highest level of total retinol was found in the liver. The control and the groundnut oil-fed groups had low levels of total retinol in the extrahepatic tissues. In the vitamin A-fed group, however, the total retinol content increased in lung, intestine, and kidney. The levels of total retinol in lung and intestine were now comparable to the levels of retinol found in the liver of normal rats.

In the vitamin A-fed group, intestine had the highest relative increase. The level of total retinol in intestine increased 355-fold as compared to the control group. The increase in the total retinol content in the other tissues were smaller; lung, kidney, and liver increased 50-fold, 25-fold, and 15-fold, respectively. These results showed that administration of vitamin A increased the level of total retinol, not only in the liver but also in extrahepatic tissues.

Tissue levels of triacylglycerol

The concentration of triacylglycerol in liver, lung, intestine, and kidney was analyzed (**Table 2**). No differences were observed in the concentration of triacylglycerol for any of the tissues in the vitamin A-fed group compared to the control group. The kidney and the intestine of the groundnut oil-fed group had larger mean values than the same tissues in the two other dietary groups. However, due to large individual variation in the groundnut oil-fed group, the differences were not significant. These results show that the groundnut oil feeding only affected the triacylglycerol levels to a small degree, and that vitamin A feeding did not increase the level of triacylglycerol in the various tissues.

Morphological staining of stellate cells by the gold chloride method

Vitamin A in lipid droplets can be identified as gold chloride precipitates. In control rats, positive gold chloride staining was seen in small, uneven structures localized in the hepatic stellate cells (**Fig. 1A**). In vitamin A-fed rats, gold chloride staining was detected in hepatic stellate cells as well as interstitial stellate cells in lung and kidney. In the lung, positive cells were localized

TABLE 2. Concentration of triacylglycerol in different tissues

Diet Group	Liver	Lung	Kidney	Intestine
	nmol/g tissue wet weight			
Control	12.7 ± 0.3	4.7 ± 0.7	7.7 ± 0.7	17.0 ± 1.5
Vitamin A	15.0 ± 2.9	3.7 ± 0.7	8.0 ± 1.0	21.3 ± 4.4
Groundnut oil	12.3 ± 1.2	5.0 ± 0.6	28.0 ± 19.0	34.7 ± 8.7

The concentration of triacylglycerol was measured as described in Materials and Methods. The results are presented as means ± SE; n = 3 in each diet group.

along the alveolus and the bronchioles (Fig. 1B). In the kidney, positive stellate cells was observed in the area between the tubules (data not shown). In neither of the dietary groups was reproducible gold chloride staining detected in sections from the intestine.

These experiments demonstrate, as expected, that vitamin A–gold chloride precipitates can be observed in liver, lung, and kidney stellate cells after feeding of high amounts of vitamin A. In the control rats, however, only hepatic stellate cells were positive. These results may, therefore, suggest that vitamin A is not stored in lipid droplets in extrahepatic stellate cells in normal rats. An alternative interpretation may be that vitamin A is stored in the lipid droplets, but that the sensitivity of the gold chloride method is too low.

Morphology using vitamin A autofluorescence

Examination of the tissue sections by fluorescence microscopy demonstrated the characteristic vitamin A fluorescence from lipid droplets in both hepatic and extrahepatic tissues from vitamin A-fed animals, but not in extrahepatic tissues from control animals. The distribution of the fluorescence corresponded to the distribution of gold chloride precipitate.

In liver sections, a strong fluorescence that reflected the distribution of hepatic stellate cells with lipid droplets containing vitamin A was seen (**Fig. 2A**). In the lung, the fluorescence appeared in small spots along the alveolus (Fig. 2B), and in the kidney, the fluorescence was detected on the outside of the tubule structures in the cortex (Fig. 2C). Finally, in the intestine, the fluorescence appeared homogeneously distributed in the lamina propria (Fig. 2D). No fluorescence was detected in the absorptive epithelial cells of the intestine. The rats were killed 3 days after the last intake of vitamin A. In the control group, fluorescence was only detected in the liver, but compared to the vitamin A-fed group, the fluorescence was much weaker.

In another set of experiments, rats fed a single dose of vitamin A (97.8 μmol retinoids as retinyl ester) were killed 2, 4, 6, 8, and 24 h after feeding, and sections were prepared for the autofluorescence method. In the rats killed after 2–8 h, a strong vitamin A fluorescence was observed in the epithelial cells. No vitamin A fluorescence was detected in the lamina propria. After 24 h, autofluorescence was detected only in the lamina propria of the intestine. In the control animals, no fluorescence was observed in the epithelial cells or in the lamina propria. These results indicate that the levels of vitamin A in the tissues from the control rats were too low to be detected by the autofluorescence method.

Immunocytochemical identification of desmin and α-smooth muscle actin positive cells

As is has been shown that hepatic stellate cells produce desmin and α-smooth muscle actin (4), we tested whether these proteins could also be identified by immunocytochemistry in extrahepatic stellate cells. Sections from liver, lung, kidney, and intestine of control animals were treated as described in Materials and Methods. As shown in **Fig. 3 A–B**, strong labeling of both desmin and α-smooth muscle actin was obtained in hepatic stellate cells. In sections from extrahepatic tissues, we also observed that stellate cells with similar localization as observed by the gold chloride method and the vitamin A autofluorescence technique were labeled with the two stellate cell markers (see Fig. 3C–G).

Morphometric quantitation of lipid droplets in liver stellate cells

It has been shown earlier that the lipid droplet size and number in hepatic stellate cells are dependent on the dietary retinoid intake (5). The retinyl ester content of the lipid droplets, but not the triacylglycerol content, is also dependent on the intake of vitamin A. Thus, the area of the lipid droplets can be used as a measure for vitamin A content.

The presence of lipid droplets in hepatic stellate cells from the control and vitamin A-fed groups was examined by transmission electron microscopy. The number of lipid droplets in hepatic stellate cells increased considerably in vitamin A-fed rats compared to control rats (**Fig. 4A–B**). The number and appearance of lipid droplets were identical in the control and groundnut-fed groups, therefore only electron micrographs from the

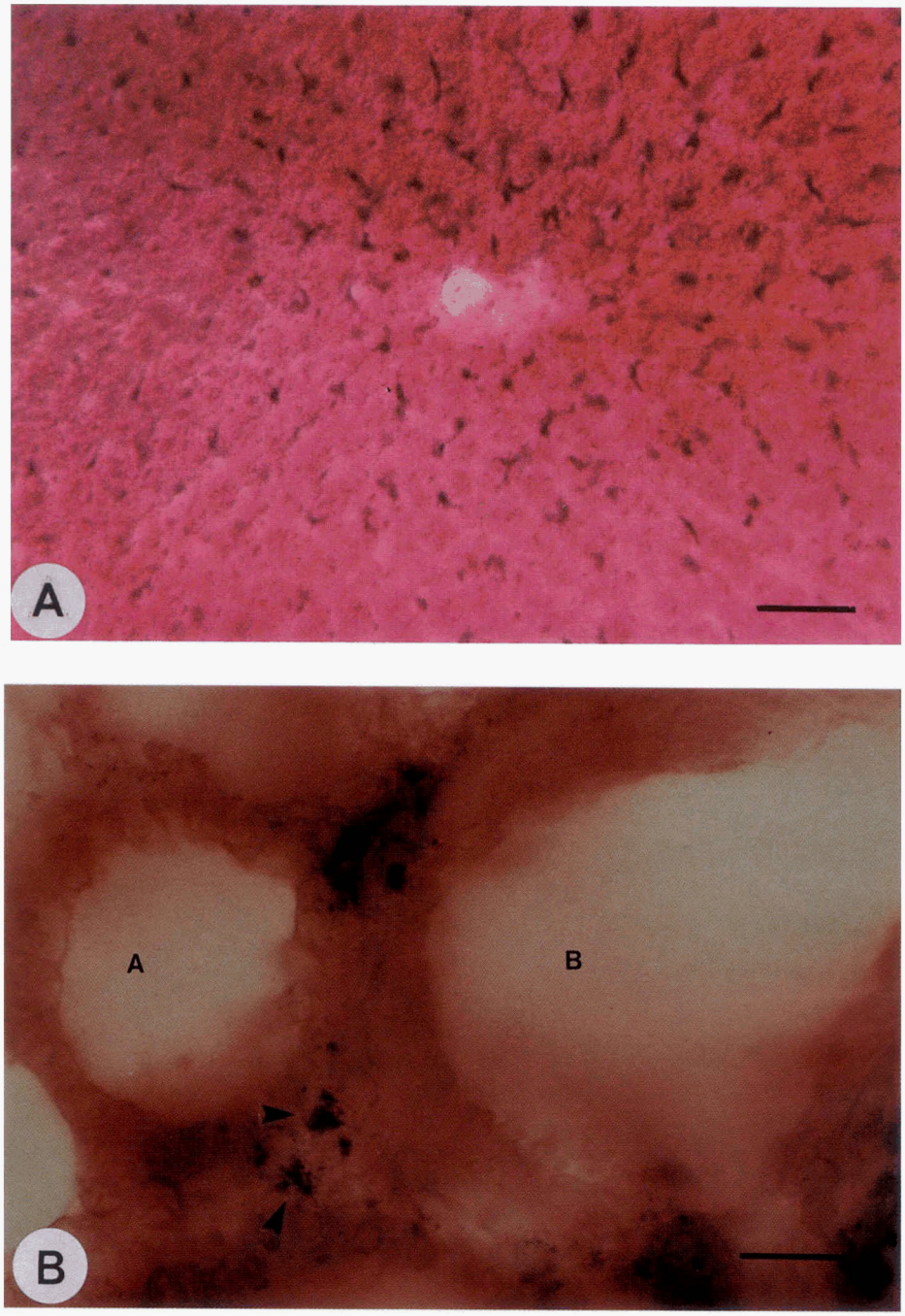


Fig. 1. Gold chloride precipitate in interstitial regions of the liver (panel A) from control rats, and lung (panel B) from vitamin A-fed rats. Formaldehyde-fixed sections were incubated with 0.01% gold chloride for 8 h. Arrowheads indicate the gold chloride stained regions; A, alveolus; B, bronchioles.

control animals are shown. In control rats, ground nut oil-fed rats, and vitamin A-fed rats, lipid droplets were also observed in the hepatic parenchymal cells, but not in endothelial or Kupffer cells (data not shown).

As the number and size of lipid droplets in stellate cells reflect the vitamin A storage in the cells, we performed morphometric quantitations of the area of lipid droplets in stellate cells from different dietary groups. In hepatic stellate cells, the total area of lipid droplets increased 10 times in the vitamin A-fed group, as com-

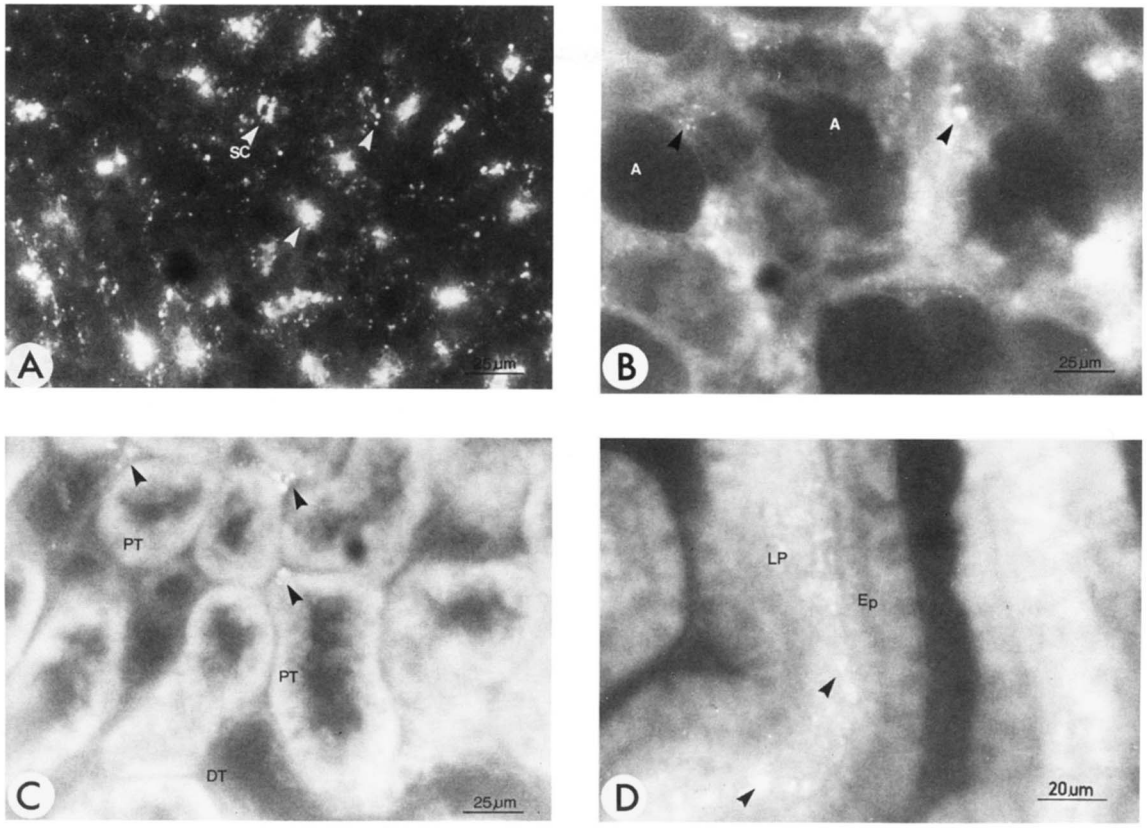


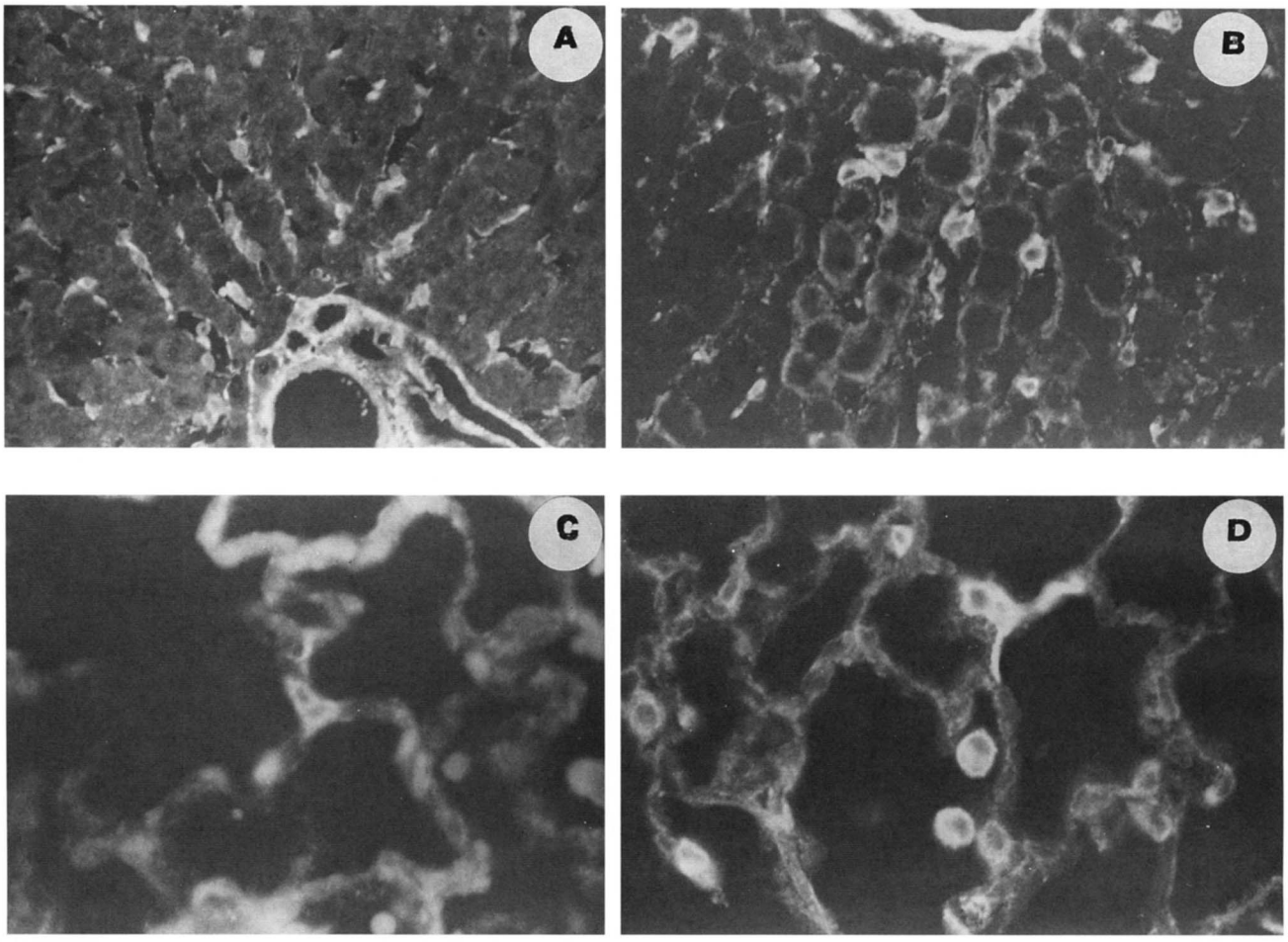
Fig. 2. Vitamin A fluorescence in interstitial regions of the liver (panel A), lung (panel B), kidney (panel C), and the small intestine (panel D) from vitamin A-fed rats (SC, stellate cell; A, alveolus; PT, proximal tubule; DT, distal tubule; LP, lamina propria; Ep, epithelia). Formaldehyde-fixed sections were studied under UV light 326 nm. Arrowheads indicate the fluorescence regions.

pared to the control and the groundnut oil-fed group ($P < 0.001$) (**Table 3**). In hepatic parenchymal cells, there was a small significant decrease in the lipid droplet area in the vitamin A-fed group compared to the control group ($P < 0.05$). In the groundnut oil-fed group there was no significant difference in the total area of lipid droplets in parenchymal cells as compared to the control group.

Morphometric quantitation of lipid droplets in lung stellate cells

Interstitial stellate cells in lung from both the control and the vitamin A-fed groups contained some lipid droplets (**Fig. 5A–B**). Similar to hepatic stellate cells, lung stellate cells were localized in association with collagen fibers and they also had long cytoplasmic projections. In lung stellate cells, the total area of lipid droplets in the vitamin A-fed group increased 2.3 times as compared to the control group ($P < 0.05$; Table 3). In vitamin A-fed animals, the total area of stellate cell lipid droplets in lung corresponded to approximately one third of the area in hepatic stellate cells. As liver contains 20 times more retinyl esters than lung in vitamin A-fed rats (Table 1), these data suggest that lipid droplets in lung stellate cells contain fewer retinyl esters than the lipid droplets in hepatic stellate cells.

The ability of rat lung to accumulate vitamin A after intake of large doses has been shown in both perinatal (16, 17) and adult lung in rats (18). The vitamin A-storing cells in the lung of rats receiving high doses of vitamin A have previously been characterized and isolated (18). The lung stellate cells were identified as vitamin A-storing cells by the specific vitamin A-fluorescence and the morphological similarity to the hepatic stellate cells with irregular form and extended cell processes. Lung stellate cells in rats fed excess vitamin A have also been characterized by the use of Sudan red-staining, gold chloride, and electron microscopy (7, 8). The lung stellate cells are localized in the subpleural tissue, in the connective tissue surrounding the bronchioles, and in the alveolar septa. In addition, several studies have suggested that retinoids are involved in lung development



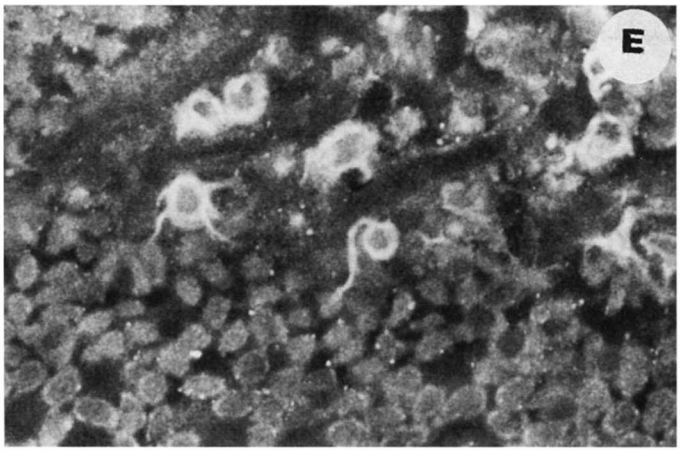
and maturation (19). It has been suggested that local storage of vitamin A has a role in lung development, thus making the fetal lung independent of the liver vitamin A stores. (19).

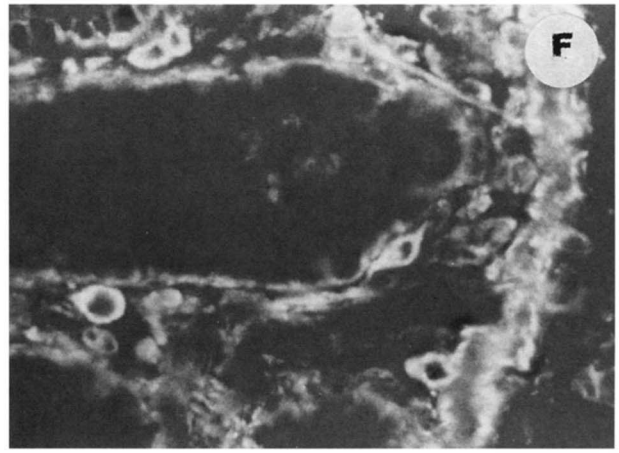
Lipid droplets in kidney stellate cells

Popper and Greenberg (12) previously identified vitamin A in kidney cortex and medulla of control rats, using the specific autofluorescence of retinyl ester. When excess vitamin A was administered, the vitamin A content of the kidney increased considerably. In our study, very few lipid droplet-containing stellate cells were observed in the interstitial regions of kidney cortex (**Fig. 6A–B**). In the control and the groundnut oil-fed rats, only 0.5 and 0.2 (mean, n = 15) lipid droplet-containing stellate cells were observed per section (5 μm^2), respectively. In the vitamin A-fed animals, 3.3 (mean, n = 15) lipid droplet-containing stellate cells were observed in the same area.

Morphometric quantitation of lipid droplets in the small intestinal stellate cells

In the intestine, lipid droplet-containing stellate cells were observed in the lamina propria from both the vitamin A-fed animals and the control animals. Rats from the vitamin A group were killed 3 days after the last feeding of vitamin A. Cells with few, small lipid droplets were observed in sections from the control group. In the vitamin A-fed group, a large increase in number of lipid droplet-containing stellate cells was observed. ((**Fig. 7A–B**). These cells appeared morphologically similar to the vitamin A-storing stellate cells from the other extrahepatic organs. It is, however, difficult to characterize these extrahepatic intestinal cells. In the lamina propria there are two types of cells with characteristics resembling those of hepatic stellate cells: smooth muscle cells and fibroblasts. Further investigation is required in order to conclude which of these cell types is involved in intestinal storage. In the intestinal





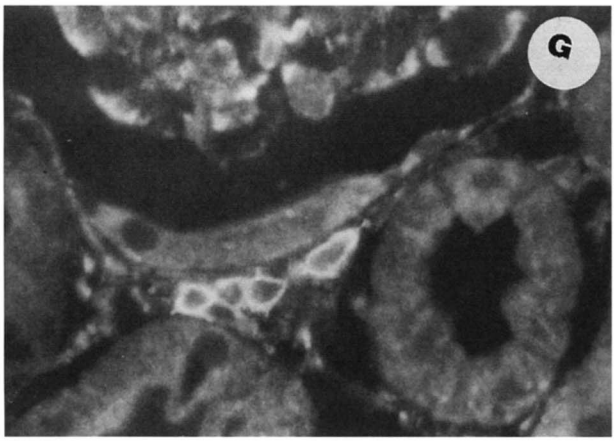


Fig. 3. Desmin (panels A, C, E) and α-smooth muscle actin (panels B, D, F, G) staining in interstitial regions of the liver (panels A, B), lung (panels C, D), the small intestine (panels E, F), and kidney (panel G) from control rats.

stellate cells, the total area of lipid droplets (Table 3) increased about 35 times in the vitamin A-fed group as compared to the control and groundnut oil-fed groups. The total stellate cell lipid droplet area from the vitamin A-fed group corresponded to approximately 68% of the total hepatic stellate cell lipid droplet area. There were no significant differences in the total stellate cell lipid droplet area in the groundnut oil-fed group as compared to the control group.

Our results are supported by previous reports that have described the existence of vitamin A-storing cells in the intestine identified by the use of Sudan red-staining, gold chloride, autofluorescence, and electron microscopy (6–8). The vitamin A-storing cells were detected in control rats as well as in vitamin A-treated rats, and were mainly localized in the lamina propria of the intestine. Morphologically the cells are similar to the hepatic stellate cells. Earlier studies have shown that

TABLE 3. Area fraction of lipid droplet in different tissues

Diet Group	Hepatic Stellate Cell	Hepatic Parenchymal Cell	Lung Stellate Cell	Intestinal Stellate Cell
		mm^2/cm^2		
Control	0.2 ± 0.04	0.8 ± 0.2	0.3 ± 0.1	0.04 ± 0.004
Vitamin A	2.2 ± 0.1***	0.2 ± 0.1*	0.7 ± 0.05*	1.5 ± 0.6**
Groundnut oil	0.2 ± 0.05	0.6 ± 0.1	0.4 ± 0.1	0.03 ± 0.01

The morphometric quantitation was performed as described in Materials and Methods. The results are presented as means ± SE; n = 3 in each diet group.

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$ vs. control rats.

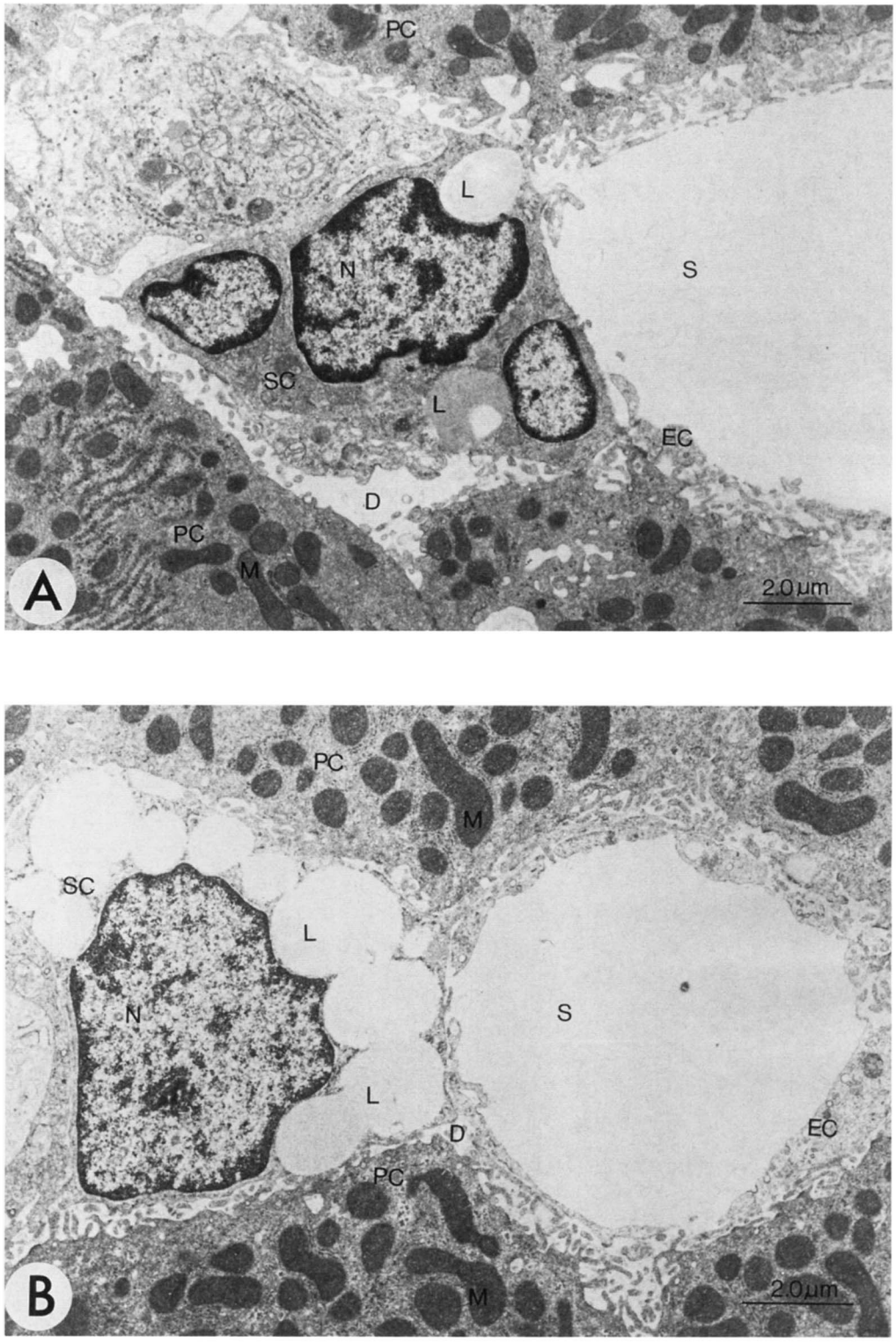


Fig. 4. Appearance of hepatic stellate cells in control (panel A) and vitamin A-fed (panel B) rats (SC, stellate cells; PC, parenchymal cells; EC, endothelial cells; L, lipids; N, nucleus; M, mitochondria; S, sinusoids; D, space of Disse). The specimen preparation for electron microscopy was performed as described in the Experimental section.

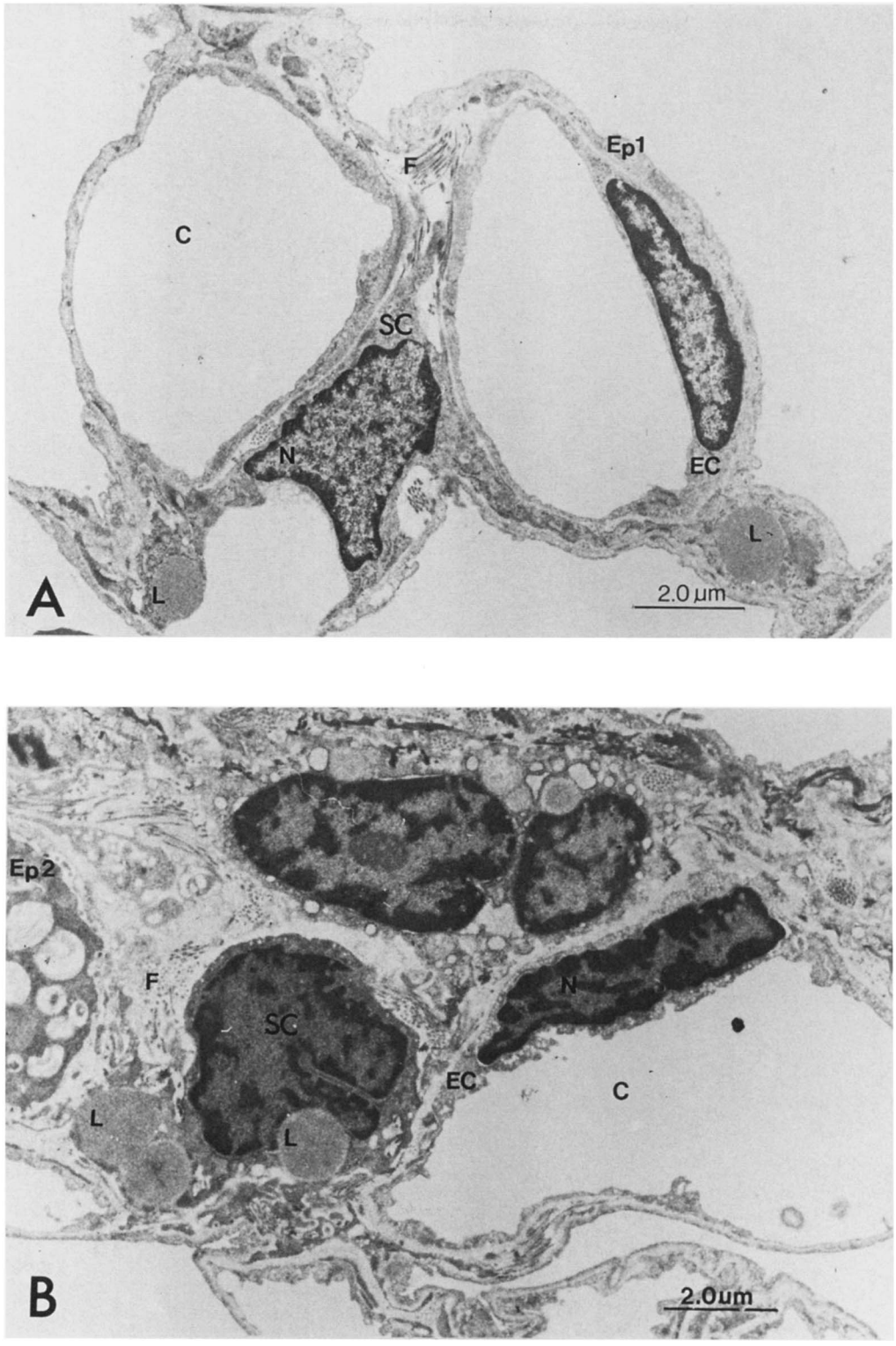


Fig. 5. Appearance of stellate cells in septa of the lung in control (panel A) and vitamin A-fed (panel B) rats (SC, stellate cells; EC, endothelial cells; Ep1, epithelial cell type 1; Ep2, epithelial cell type 2; L, lipids; N, nucleus; F, collagen fibres; C, capillary). The specimen preparation for electron microscopy was performed as described in the Experimental section.

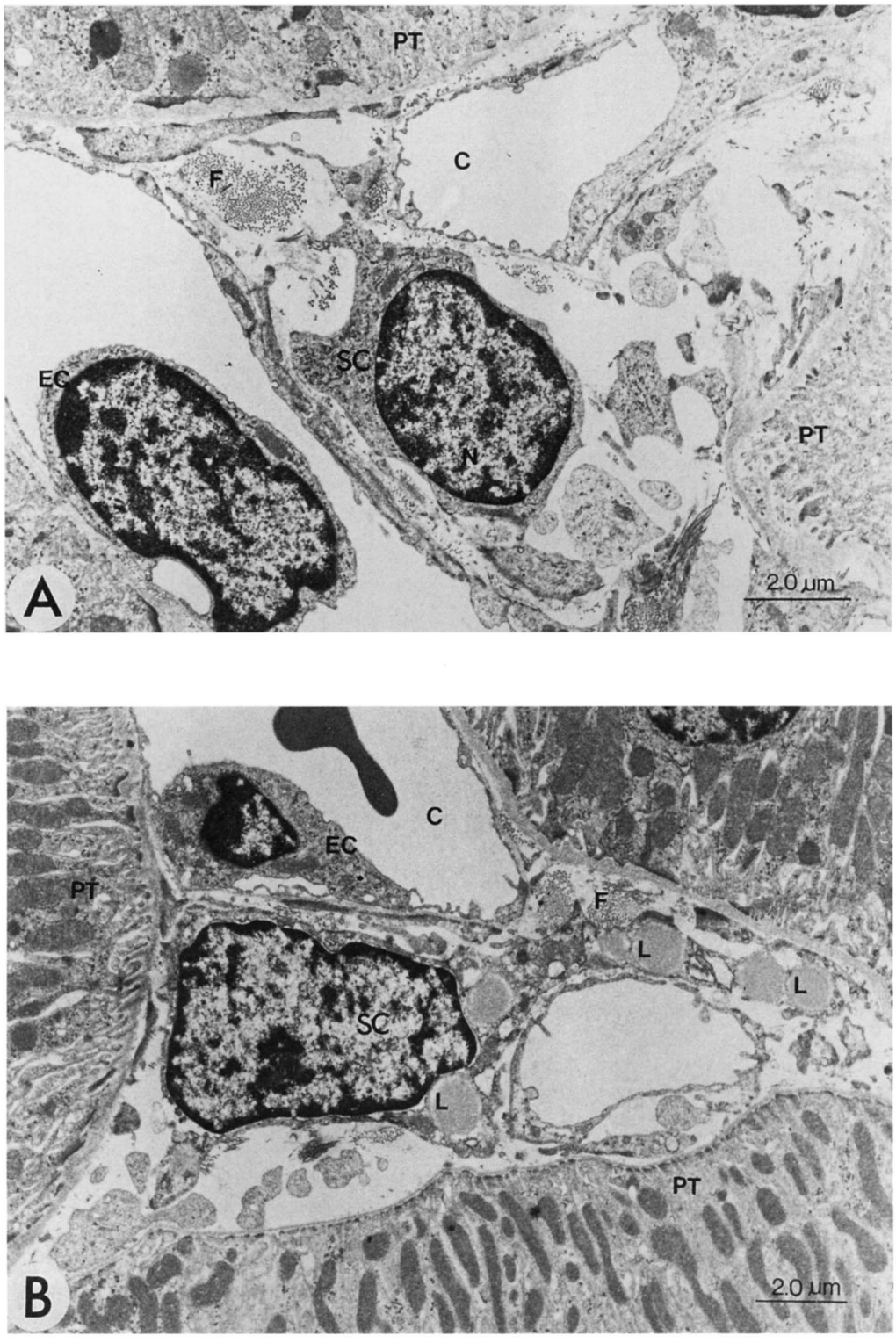


Fig. 6. Appearance of stellate cells in the cortex interstitium of the kidney in control (panel A) and vitamin A-fed (panel B) rats (SC, stellate cells; EC, endothelial cells; PT, proximal tubule cells; L, lipids; N, nucleus; F, collagen fibres; C, capillary). The specimen preparation for electron microscopy was performed as described in the Experimental section.

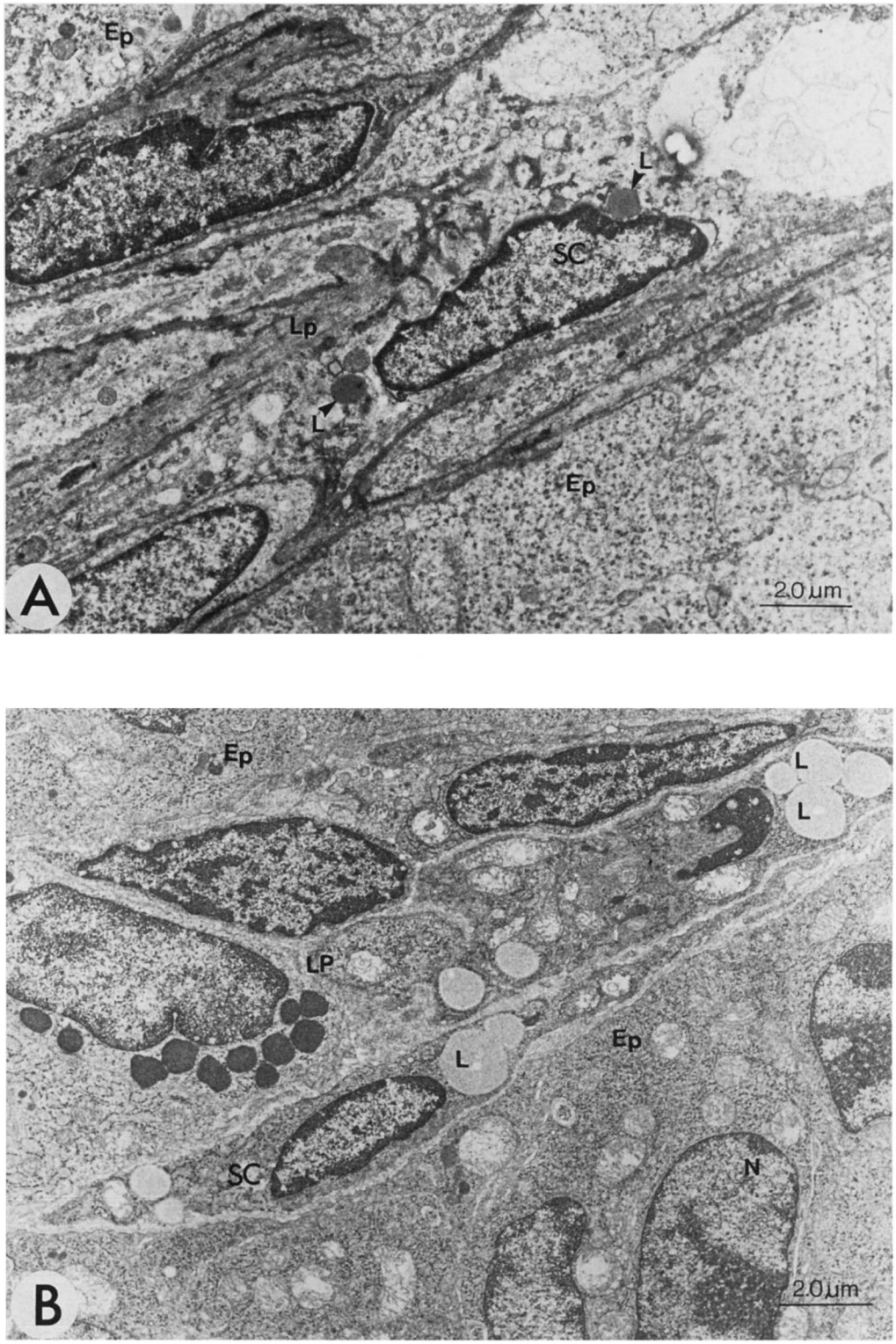


Fig. 7. Appearance of stellate cells in the lamina propria of the small intestine in control (panel A) and vitamin A-fed (panel B) rats (SC, stellate cells; Ep, epithelia; LP, lamina propria; L, lipids; N, nucleus). The specimen preparation for electron microscopy was performed as described in the Experimental section.

both lung epithelial cells as well as the intestinal epithelial cells are particularily vulnerable to vitamin A depletion, and a hypothesis is that local storage of vitamin A is needed in order to maintain these tissues (20, 21).

In summary, lipid droplet-containing stellate cells were identified in liver, lung, kidney, and intestine in normal as well as in vitamin A-fed rats. In rats fed the control diet, the stellate cell lipid droplet areas in liver, lung, and intestine constitute about 0.02%, 0.03%, and 0.004% of the total tissue area, respectively. When the rats were administered excess vitamin A, the hepatic, lung, and intestinal stellate cells lipid droplet areas increased about 10-fold, 2-fold, and 40-fold, respectively. In contrast to hepatic stellate cells, stellate cells in the extrahepatic tissues show specific vitamin A fluorescence and positive gold chloride reaction only after administration of excess vitamin A.

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