Metabolism and secretion of retinol transport complex in acute renal failure

Thomas H. Gerlach and Maija H. Zile

Department of Food Science and Human Nutrition, Michigan State University, East Lansing, MI 48824-1224

Abstract Tissue uptake and distribution of retinol from circulatory vitamin A transport complex was studied in order to determine the origin of the increased serum retinol in rats with short-term acute renal failure. In rats with acute renal failure, serum retinol increased 37–70% within 2 h after surgery. After an injection of donor plasma containing 1.8 μCi of [^{3}H]retinol in retinol transport complex, in rats with renal failure the ability to clear radioactivity was decreased 36% by 0.5 h and 57% by 2 h, as compared to sham-operated rats. The uptake and distribution of radioactivity by nonrenal tissues was similar in rats with acute renal failure and with intact kidneys. The lack of renal function did not alter hepatic cycling of [^{3}H]retinol from the circulation and thus could not account for the increased serum retinol in renal failure. When hepatic release of retinol-retinol binding protein was blocked by colchicine, the up-regulation of serum retinol, normally observed in rats with acute renal failure, was abolished. Our studies provide strong evidence that kidney has an important role in maintaining serum retinol homeostasis by influencing the release of retinol-retinol binding protein from liver into circulation. Peripheral tissue uptake of circulatory retinol and hepatic cycling of nonutilized retinol are not directly influenced by the kidney.—**Gerlach, T. H., and M. H. Zile.** Metabolism and secretion of retinol transport complex in acute renal failure. *J. Lipid Res.* 1991. **32:** 515–520.

Supplementary key words retinol • retinol binding protein • hepatic release of retinol

Vitamin A is transported from its hepatic stores to peripheral target sites in the form of retinol bound to its specific carrier protein, retinol binding protein (RBP) and transthyretin (TTR) (1–3). After delivery of retinol to target tissues, the complex dissociates, apoRBP is filtered by the glomeruli, and the nonutilized retinol in the retinol-RBP-TTR complex is recycled to the liver (4–6). The circulatory level of retinol is homeostatically controlled and remains relatively constant (7). The mechanism(s) regulating circulatory retinol homeostasis is not known.

We have recently observed an increase in circulatory retinol in rats with experimental acute renal failure (8, 9) and have established that this increase is almost entirely due to retinol in the retinol-RBP-TTR complex (9) and that this retinol is derived from the hepatic pool of retinol newly acquired from the diet, suggesting that the kidney modulates release of this retinol (9). It is not clear, however, whether, due to renal failure, a change in peripheral tissue uptake or in hepatic cycling of retinol from its circulatory transport complex had contributed to expansion of the circulatory retinol pool.

In the present studies we continue to elucidate the role of the kidney in circulatory vitamin A homeostasis. Utilizing a short-term acute renal failure model, we examined the release of retinol-RBP from the liver in the presence of colchicine, a drug known to interfere in the transport of secretory vesicles from Golgi apparatus to the plasma membrane (10) and shown by Smith and coworkers (11) to inhibit RBP secretion. Our findings provide evidence that the kidney influences hepatic release of retinol-RBP and that peripheral utilization of circulatory retinol is not altered by the short-term absence of renal function.

MATERIALS AND METHODS

Chemicals

Sephadex G-150 superfine was purchased from Pharmacia Inc., Piscataway, NJ. Xylasine (Rompun) was obtained from Mobay Corp., Shawnee, NY, and ketamine (Ketaset) was obtained from Bristol Labs, Syracuse, NY. Soluene 350 and scintillation cocktail were purchased from Packard Instruments, Downers Grove, IL. Molecular weight markers, buffers, and colchicine were purchased from Sigma, St. Louis, MO.

Animals and diets

Male Sprague-Dawley rats (Harlan Sprague Dawley Inc., Indianapolis, IN and Holtzmann Co., Madison,

Abbreviations: RBP, retinol binding protein; TTR, transthyretin; HPLC, high pressure liquid chromatography.

WI) were used. The rats were housed in an environmentally controlled room with 12 h of light, at 24°C and 40% relative humidity. Depending on the treatment group, water and vitamin A-deficient semisynthetic (12) or stock diet (Wayne Rodent Blox, Continental Feed Co., Wayne, IL) was fed ad libitum.

Retinoids; retinoid analysis

All-*trans*-retinyl acetate and all-*trans*-retinol were gifts from Hoffmann-LaRoche, Nutley, NJ. The purity of all retinoids was checked by high pressure liquid chromatography (HPLC). When necessary, the retinoids were purified. [11,12(N)-^{3}H]retinol, (all *trans*) (50.2 Ci/mmol), was purchased from New England Nuclear-DuPont, Wilmington, DE; it was found to be 97% pure. Retinoids in biological samples were extracted and analyzed by HPLC as described previously (9, 13).

Surgical procedures

Acute renal failure in rats was produced by exposing the kidneys via flank incisions, followed by bilateral ligation of kidney arteries and veins. For sham operation only the flank incisions were performed. Surgery was performed under combination anesthesia of xylasine, 6 mg/kg of body weight, and ketamine, 80 mg/kg body weight. At the end of the experiments rats were anesthetized and killed by cardiac puncture.

Preparation of [^{3}H]retinol-RBP-TTR

Plasma containing tritium-labeled retinol-RBP-TTR was prepared as described previously (4), using the knowledge that the administration of vitamin A to vitamin A-depleted rats results in the release of hepatic retinol-RBP into circulation (4, 14, 15). Briefly, nine vitamin A-depleted rats, prepared as described earlier (9), were each given 100 μCi of [^{3}H]retinol in 0.1 ml of corn oil by gastric intubation. To increase the amount of retinol-RBP-TTR in circulation, acute renal failure was induced (see Surgical procedures) in the rats 3 h after the administration of labeled retinol (9). The rats were killed 2 h after the surgery, and their serum (donor serum) was obtained, pooled, and stored under N_2 at −76°C until use. The donor serum contained 2 μCi/ml; 97% of the serum radioactivity was in the retinol fraction (see Retinoid analysis).

Donor serum was given to recipient rats within 7 days of collection. To verify that the labeled retinol was carried on RBP-TTR, donor serum was chromatographed on a 62 × 1.5 cm Sephadex G-150 column that had been calibrated with molecular weight markers (9). Proteins were eluted at a flow rate of 4.4 ml/h using 0.02 M potassium phosphate buffer, pH 7.4, containing 0.2 M NaCl. Fractions were assayed for radioactivity and for fluorescence characteristic to vitamin A compounds (16), using excitation at 334 nm and emission at 465 nm (Varian SF-330 Spectrofluorimeter, Varian Associates, Inc., Palo Alto, CA). Most (> 93%) of the radioactivity associated with retinol was in the retinol-RBP-TTR complex fraction.

Experimental protocols

For the study of [^{3}H]retinol-RBP-TTR metabolism, rats fed a stock diet were obtained at the age of 11 weeks, weight-matched (300–350 g), divided into two groups of 12 rats each, and treated as follows. One group of rats was subjected to ligation of renal arteries and veins (see Surgical procedures) and immediately thereafter injected i.v. with 0.9 ml of donor serum containing [^{3}H]retinol-RBP-TTR, 1.8 μCi, and 0.5 μg of retinol (see above). The second group of rats was sham-operated and injected with donor serum as above. All rats were fasted overnight before experiments. Rats were killed 0.5, 1, and 2 h after the above treatments. Blood was removed by cardiac puncture and centrifuged to remove cells and obtain serum. Liver, lungs, spleen, kidneys, and testes were removed. Liver was perfused, and the intestine was flushed with cold saline to remove contents. Urine was obtained from the bladder. All biological material was stored at −76°C.

The effect of colchicine on renal failure-induced elevation of serum retinol was studied in 12-week-old vitamin A-adequate rats maintained on stock diet. Ten rats were given an i.p. injection of colchicine, 5 mg/kg body weight, in 0.5 ml of 0.9% NaCl, and 10 control rats were injected with 0.9% NaCl. After 3 h 5 rats from each treatment group were either sham-operated or their renal arteries and veins were ligated as described in Surgical procedures. Blood samples were removed from the tail vein 1 h after surgery; the rats were killed 2 h after surgery. Serum retinol was analyzed by HPLC (9, 13).

Preparation of samples for radioactivity measurements

Tissues were minced with a razor blade into small pieces, mixed to obtain a homogenous distribution, 0.5 g was transferred to scintillation vials and digested with 2 ml of Soluene by incubation in a shaking water bath at 60°C for 12 h. The digested samples were decolorized with 30% of hydrogen peroxide, neutralized with 0.5 ml of 0.2 N HCl, mixed with 15 ml of scintillation cocktail, and counted in a model 4430 Packard Scintillation counter (Packard Instruments). Serum, 0.1 ml, was digested with 0.2 ml of Soluene for 1 h, then processed as above.

Other methods

All procedures were conducted under gold fluorescence lights. All chemicals and solvents used were reagent or HPLC grade. Serum volume was calculated on basis of body weight, as follows: body weight in grams × 0.035 = estimated serum volume (17). Statistically significant

changes were defined as $P < 0.05$ as determined by Student's t-test (18).

RESULTS

Serum retinol concentration after induction of acute renal failure

Fig. 1 illustrates the effect of acute renal failure on serum retinol homeostasis. As early as 0.5 h after ligation of renal arteries and veins, serum retinol concentration was increased by 37% compared to sham-operated rats; an increase of 48% was evident 1 h after induction of renal failure and reached 70% after 2 h. Serum retinol decreased slightly (from 29.3 to 26.1 μg/dl) in sham-operated rats over this 2-h time period.

Clearance of [^{3}H]retinol-RBP-TTR from circulation

Fig. 2 shows the time course of clearance of injected [^{3}H]retinol-RBP-TTR from the circulation of rats with acute renal failure and sham-operated controls. Intact rats had cleared 48% of the radioactivity from injected [^{3}H]retinol-RBP-TTR within 0.5 h, while rats with acute renal failure were able to clear only 20% of the injected radioactivity. The reduced ability of rats with renal failure to remove the injected dose from the circulation was evident throughout the 2-h time course: at 0.5 h the clearance was reduced by 36%, at 1 h, by 38% and at 2 h, by 57% compared to sham-operated controls.

Time course of distribution of radioactivity in tissues

Table 1 illustrates the distribution of radioactivity after the i.v. injection of [^{3}H]retinol-RBP-TTR into rats with acute renal failure and into sham-operated rats. Among the tissues examined in the sham-operated rats, kidneys accumulated the greatest amount of radioactivity, reaching 27.4% within 2 h after administration of the dose. Although renal arteries and veins had been ligated, a small amount (1.8%) of dose radioactivity was found after 2 h in the kidneys of these rats.

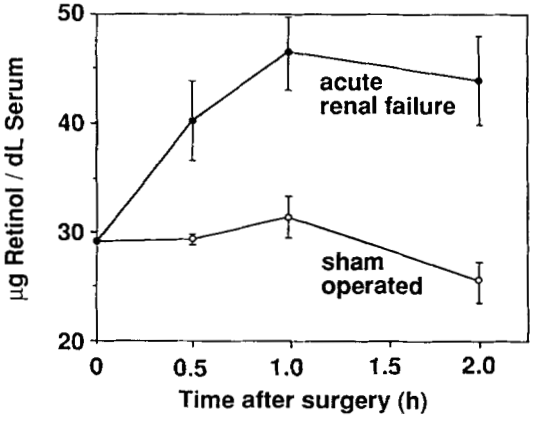

Fig. 1. Serum retinol concentration after induction of acute renal failure. Error bars represent SEM; n = 4. Values were significantly different between the experimental groups at $P < 0.05$.

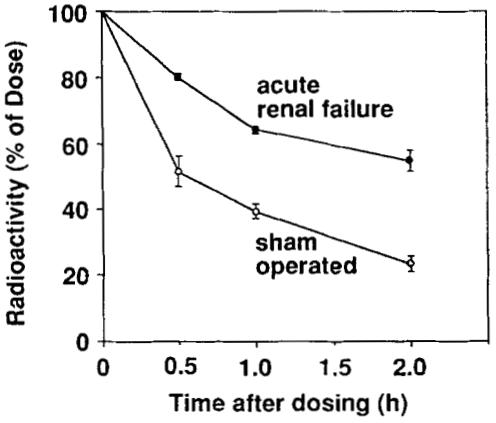

Fig. 2. Clearance of [^{3}H]retinol-RBP-TTR from circulation. Donor serum, 0.9 ml, containing 1.8 μCi of [^{3}H]retinol in the retinol-RBP-TTR complex, was injected i.v. and the time course of disappearance of radioactivity from circulation was determined in rats with acute renal failure and in sham-operated controls. Error bars represent SEM; n = 4. Values were significantly different between the experimental groups at $P < 0.05$.

While there were no differences in the liver radioactivity between sham-operated and renal artery/vein-ligated rats at 0.5 and 1 h after administration of the dose, significantly less (20%) radioactivity was found in the livers of the rats with acute renal failure by 2 h. Radioactivity in individual peripheral tissues accounted for 0.5–1.4% of the injected dose; there were no significant differences between rats with renal failure and their sham-operated controls. Urinary excretion in sham-operated rats accounted for 0.1–0.9% of the injected dose radioactivity by 2 h.

Distribution of radioactivity among hepatic retinoids

Table 2 illustrates the distribution of liver radioactivity among various retinoid pools. There were no significant differences in the distribution of radioactivity in the different retinoid fractions between rats with renal failure and sham-operated controls. At all time points examined most of the liver radioactivity (90–97%) in both treatment groups was in the form of retinyl esters; the retinol pool contained 3–9% of hepatic radioactivity. Over the 2-h period studied there was a gradual (but not significant) increase in the radioactivity in the retinyl ester fraction and a gradual (but not significant) decrease in the radioactivity in the retinol fraction.

Time course of distribution of radioactivity in kidneys

The distribution of radioactivity among renal retinoids in sham-operated rats is illustrated in **Fig. 3.** After the i.v.

TABLE 1. Time course of distribution of injected radioactivity in tissues

Organ	Treatment[1]	% of Injected Radioactivity in Total Organ		
		After 0.5 h	After 1 h	After 2 h
Kidney	Acute renal failure	2.7 ± 0.7[a]	1.5 ± 0.7[a]	1.8 ± 0.3[a]
Kidney	Sham-operated	14.7 ± 0.8	20.1 ± 1.0	27.4 ± 1.5
Liver	Acute renal failure	7.0 ± 0.2	7.6 ± 0.3	8.2 ± 0.4[a]
Liver	Sham-operated	6.3 ± 0.5	7.1 ± 0.5	10.3 ± 0.5
Intestine	Acute renal failure	1.3 ± 0.2	1.8 ± 0.2	2.0 ± 0.1
Intestine	Sham-operated	1.0 ± 0.1	1.4 ± 0.1	1.9 ± 0.3
Lung	Acute renal failure	1.0 ± 0.1	1.1 ± 0.1	1.4 ± 0.2
Lung	Sham-operated	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.1
Testis	Acute renal failure	0.5 ± 0.1	0.7 ± 0.1	1.0 ± 0.2
Testis	Sham-operated	0.5 ± 0.1	0.7 ± 0.1	0.7 ± 0.2
Spleen	Acute renal failure	0.7 ± 0.1	0.3 ± 0.0	0.3 ± 0.0
Spleen	Sham-operated	0.9 ± 0.4	0.3 ± 0.0	0.3 ± 0.1
Urine	Acute renal failure			
Urine	Sham-operated	0.1 ± 0.0	0.9 ± 0.1	0.1 ± 0.0

After renal surgery, rats were injected intravenously with 1.8 μCi of [^{3}H]retinol-RBP-TTR in 0.9 ml of donor serum and killed at different time points thereafter. Experimental details are described in Materials and Methods. Values are expressed as means ± SEM; n = 4.

[a]Significantly different from sham-operated at $P < 0.05$.

injection of [^{3}H]retinol-RBP-TTR, within 0.5 h most of renal radioactivity (59%) was in retinol fraction, while 30 and 12% were in retinyl esters and polar retinoids, respectively. Although radioactivity in the retinyl ester fraction gradually increased, a relatively large amount of radioactivity sequestered by the kidney (47%) remained in the retinol fraction 2 h after the i.v. administration of the radioactive retinol transport complex. Radioactivity in the polar retinoids, however, remained relatively constant over the 2-h time period examined.

TABLE 2. Distribution of radioactivity among hepatic retinoids

Time after Treatment	Treatment	% of Liver Radioactivity in		
		Polar Retinoids	Retinol	Retinyl Esters
h				
0.5	Acute renal failure	1.1 ± 0.3	9 ± 2	90 ± 2
0.5	Sham-operated	1.6 ± 0.3	8 ± 2	91 ± 1
1	Acute renal failure	1.4 ± 0.3	6 ± 1	93 ± 1
1	Sham-operated	2.0 ± 0.8	6 ± 0	92 ± 1
2	Acute renal failure	0.5 ± 0	5 ± 1[a]	95 ± 1
2	Sham-operated	0.7 ± 0.2	3 ± 0	97 ± 0

Rats were either sham-operated or subjected to ligation of renal arteries and veins. Immediately after surgery, they were injected intravenously with 1.8 μCi of [^{3}H]retinol-RBP-TTR in 0.9 ml of donor serum and killed at various time points thereafter. Retinoids and radioactivity were determined as described in Materials and Methods. Values are expressed as means ± SEM; n = 4.

[a]Significantly different from sham-operated at $P < 0.05$.

Effect of colchicine

The effects of colchicine administration prior to the induction of renal failure are shown in **Fig. 4**; they were most prominent 2 h after surgery. While the induction of renal failure resulted in the expected rise in serum retinol (71% above sham-operated controls), in the rats given col-

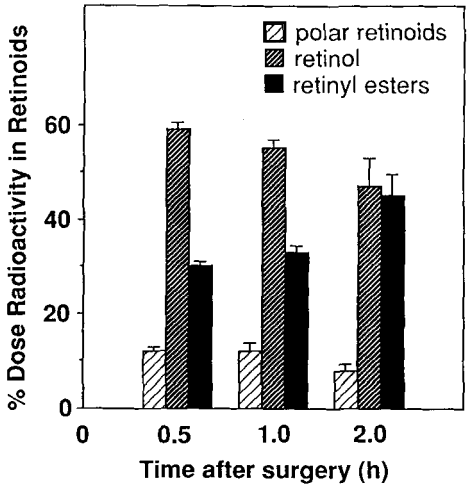

Fig. 3. Time course of distribution of radioactivity in kidney after i.v. injection of [^{3}H]retinol-RBP-TTR. Sham-operated rats were injected i.v. with 0.9 ml of donor serum containing 1.8 μCi of [^{3}H]retinol-RBP-TTR. Rats were killed at different time points. Retinoids and radioactivity were determined as described in Methods. Error bars represent SEM; n = 4.

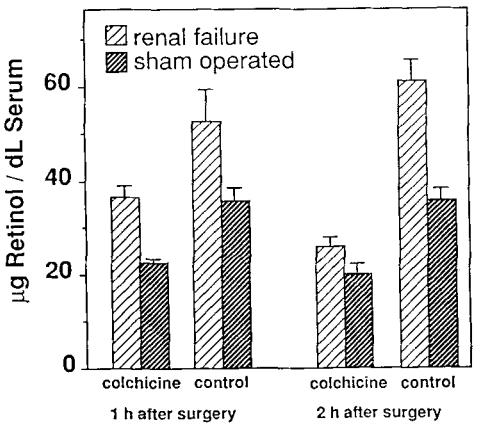

Fig. 4. Effect of colchicine on serum retinol concentration in acute renal failure. Rats were injected with colchicine (5 mg/kg body wt) 3 h before surgery and serum retinol concentration was determined 1 and 2 h after surgery. Error bars represent SEM; n = 4–5.

chicine prior to renal surgery, the rise in serum retinol concentration was only 29%. In both groups of rats colchicine treatment resulted in the lowering of serum retinol; in sham-operated rats colchicine depressed serum retinol concentration from 36 to 20 µg/dl; in rats with acute renal failure colchicine treatment depressed serum retinol concentration from 61 to 26 µg/dl (58% decrease), a retinol value lower than that of intact untreated rats.

Vitamin A content of liver and kidneys

Liver vitamin A status was determined to ascertain that rats of adequate and similar vitamin A status had been used for the study. Vitamin A values (µg/g wet weight) were as follows: liver:retinyl esters, 830 ± 185; retinol, 22 ± 0.8; kidneys:retinol, 1.6 ± 0.8; retinyl esters, 2.4 ± 1.2. Both in the liver and kidneys, no significant differences were found in retinoid concentration at the different time points examined.

DISCUSSION

As expected, serum retinol concentration was increased in rats with acute renal failure, a significant increase (37%) evident as early as 0.5 h after surgery and reaching a maximum of 70% by 2 h. The uptake of radioactivity from circulatory [^{3}H]retinol-RBP-TTR by the peripheral tissues examined in this study was not altered by short-term acute renal failure and thus cannot be responsible for the increased retinol-RBP-TTR that we routinely observed in rats with short-term acute renal failure (8, 9). Similarly, hepatic uptake and distribution of radioactivity from circulatory [^{3}H]retinol-RBP-TTR into the various hepatic retinoid pools was not significantly different in rats with intact kidney function and those with short-term acute renal failure 0.5 and 1 h after the injection of the labeled retinol-RBP-TTR when serum retinol concentration was already significantly increased. These findings provide evidence that hepatic cycling of retinol from the serum pool is not altered in short-term acute renal failure. The elimination of the above mechanisms as being responsible for the elevated serum retinol in renal failure strengthens our previous data and the proposed hypothesis that the increase of retinol in the circulatory retinol-RBP-TTR complex in acute renal failure is associated with an enhanced release of hepatic retinol-RBP, using retinol from the newly acquired dietary vitamin A pools (9). In support of different hepatic retinol pools is our observation that [^{3}H]retinol from the circulatory [^{3}H]retinol carrier complex, upon cycling to the liver, is very rapidly diverted to hepatic vitamin A storage sites, since as early as 30 min after injecting the radiolabeled transport complex into circulation, most of hepatic radioactivity (90%) is in the form of retinyl esters. Short-term acute renal failure did not influence this course of events.

Our previous work provided indirect evidence that acute renal failure causes an up-regulation of hepatic release of retinol into the circulation (9). Direct support for the hypothesis that renal control of circulatory retinol homeostasis is at the level of release of hepatic retinol-RBP into circulation was obtained in the present studies by treating rats with colchicine, a drug known to inhibit the secretion/release of hepatic retinol-RBP (11): the expected rise in serum retinol concentration in rats with renal failure was much smaller when these rats were pretreated with colchicine.

The use of radiolabeled vitamin A transport complex in the experimental model for acute renal failure has enabled us to reveal important aspects of hepatic and renal roles in vitamin A homeostasis that function independently from each other, as evidenced by the unchanged peripheral handling of circulatory retinol and the undisturbed hepatic handling of cycled retinol in short-term renal failure.

It is well established that the kidney has a major role in the processing of retinol transport complex (1, 2, 19, 20); our studies provide additional support for this concept. In intact rats approximately half of the radioactivity from injected [^{3}H]retinol-RBP-TTR was removed from circulation within 30 min, while only 20% of it was removed by rats with kidney failure. We observed that intact kidneys rapidly (within 30 min) sequester a large fraction (14%) of the retinol from the circulatory complex, retaining as much as a half of it in the form of free retinol and eliminating only a small amount (<1%) in urine. The large amount (27%) of dose radioactivity found in the kidneys of sham-operated rats within 2 h might suggest that in the absence of normal kidney function more retinol is retained in circulation, and this could explain the rise in serum retinol in rats with renal failure. This, however, is not the case. While renal processing of circulatory retinol is well established (1), it is also well known that

the kidney does not accumulate vitamin A (2); this was confirmed in the present study. Furthermore, by 2 h after the dosing the kidneys had taken up 27% of the injected [^{3}H]retinol, representing 27% of serum retinol pool or 0.8 μg, an amount of retinol insufficient to increase serum retinol concentration by 70% (or by a total of 1.8 μg), an increase we observed in rats 2 h after induced renal failure. Since the kidney has the potential for RBP synthesis (21, 22), it is possible that recycled renal retinol is released into circulation as retinol-RBP. The present work indicates that kidneys have a major role in handling circulatory retinol, and that hepatic and other nonrenal tissue deposition of retinol radioactivity from the circulatory retinol transport complex is handled independently of renal function.

From the present experiments we conclude that the increased retinol in the circulation of rats with acute kidney failure is not due to an altered metabolism of retinol-RBP-TTR complex by nonrenal tissues. We provide strong evidence that the increase in circulatory retinol in acute renal failure is the result of the release of retinol-RBP from the liver. Thus, normal kidney function influences the release of hepatic retinol into circulation and contributes to circulatory vitamin A homeostasis.

Supported by USDA grant 89-37200-4424 and by Michigan State University NIH Biomedical Research Funds. Acknowledgment is made to the Michigan Agricultural Experiment Station for their support of this research. The authors are grateful to Dr. Kevin Lewis for many helpful suggestions and for a critical evaluation of the results, and Dr. Dale Romsos for a critical review of the manuscript.

Manuscript received 11 September 1990.

REFERENCES

1. Goodman, D. S. 1984. Plasma retinol-binding protein. *In* The Retinoids. Vol. 2. M. B. Sporn, A. B. Roberts, and D. S. Goodman, editors. Academic Press, New York. 42–88.
2. Wolf, G. 1984. Multiple functions of vitamin A. *Physiol. Rev.* **64:** 873–937.
3. Peterson, P. A., S. F. Nilsson, L. Ostberg, L. Rask, and A. Vahlquist. 1974. Aspects of metabolism of retinol-binding protein and retinol. *Vitam. Horm.* **32:** 181–214.
4. Lewis, K. C., M. H. Green, and B. A. Underwood. 1981. Vitamin A turnover in rats as influenced by vitamin A status. *J. Nutr.* **111:** 1135–1164.
5. Blomhoff, R., K. R. Norum, and T. Berg. 1985. Hepatic uptake of [^{3}H]retinol bound to the serum retinol binding protein involves both parenchymal and perisinusoidal stellate cells. *J. Biol. Chem.* **260:** 13571–13575.
6. Gjoen, T., T. Bjerkelund, H. K. Blomhoff, K. R. Norum, T. Berg, and R. Blomhoff. 1987. Liver takes up retinol-binding protein from plasma. *J. Biol. Chem.* **262:** 10926–10930.
7. Underwood, B. A., J. D. Loerch, and K. D. Lewis. 1979. Effects of dietary vitamin A deficiency, retinoic acid and protein quantity and quality on serially obtained plasma and liver levels of vitamin A in rats. *J. Nutr.* **109:** 796–806.
8. Ikegami, S., and M. Zile. 1989. Plasma vitamin A in nephrectomized rats. Proceedings of The 14th International Congress of Nutrition, Seoul, Korea. (Abstract).
9. Gerlach, T. H., and M. H. Zile. 1990. Upregulation of serum retinol in experimental acute renal failure. *FASEB J.* **4:** 2511–2517.
10. Stein, O., L. Sanger, and Y. Stein. 1974. Colchicine-induced inhibition of lipoprotein and protein secretion into the serum and lack of interference with secretion of biliary phospholipids and cholesterol by rat liver in vivo. *J. Cell Biol.* **62:** 90–103.
11. Smith, J. E., D. D. Deen, Jr., D. Sklan, and D. S. Goodman. 1980. Colchicine inhibition of retinol-binding protein secretion by rat liver. *J. Lipid Res.* **21:** 229–237.
12. Report of the American Institute of Nutrition Ad Hoc Committee on Standards for Nutritional Studies. 1977. *J. Nutr.* **107:** 1340–1348.
13. Cullum, M. E., and M. H. Zile. 1986. Quantitation of biological retinoids by high pressure liquid chromatograph: primary internal standardization using tritiated retinoids. *Anal. Biochem.* **153:** 23–32.
14. Muto, Y., J. E. Smith, P. O. Milch, and D. S. Goodman. 1972. Regulation of retinol-binding protein metabolism by vitamin A status in the rat. *J. Biol. Chem.* **247:** 2542–2550.
15. Smith, J. E., Y. Muto, P. O. Milch, and D. S. Goodman. 1973. The effects of chylomicron vitamin A on the metabolism of retinol-binding protein in the rat. *J. Biol. Chem.* **248:** 1544–1549.
16. McGuire, B. W., and F. Chytil. 1980. Three-step purification of retinol-binding protein from rat serum. *Biochim. Biophys. Acta.* **621:** 324–331.
17. Ringler, D. H., and L. Dabich. 1979. Hematology and clinical biochemistry. *In* The Laboratory Rat. Vol. 1. J. J. Baker, J. R. Lindsey, and S. H. Weisbroth, editors. Academic Press, New York. 105–121.
18. Darlington, R. B. 1975. Radicals and squares. *In* Statistical Methods for the Behavioral Sciences. Logan Hill Press, New York. 229–503.
19. Rask, L., H. Anundi, J. Bohme, U. Eriksson, A. Frederiksson, S. F. Nilsson, H. Ronne, A. Vahlquist, and P. A. Peterson. 1980. The retinol-binding protein. *Scand. J. Clin. Lab. Invest.* **40:** 45–61.
20. Vahlquist, A., P. A. Peterson, and L. Wilbell. 1973. Metabolism of vitamin A transporting protein complex. I. Turnover studies in normal persons and in patients with chronic renal failure. *Eur. J. Clin. Invest.* **3:** 352–362.
21. Makover, A., D. R. Soprano, M. L. Wyatt, and D. S. Goodman. 1989. Localization of retinol-binding protein messenger RNA in the rat kidney and in perinephric fat tissue. *J. Lipid Res.* **30:** 171–180.
22. Soprano, D. R., K. J. Soprano, and D. S. Goodman. 1986. Retinol-binding protein messenger RNA levels in the liver and the extrahepatic tissues of the rat. *J. Lipid Res.* **27:** 166–171.