Colchicine inhibition of retinol-binding protein secretion by rat liver[1]

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Abstract Studies were conducted to explore the effects of colchicine on the secretion and metabolism of retinol-binding protein (RBP) by the liver. In the vitamin A-deficient rat, the rate of secretion of RBP from the liver into the serum is greatly reduced, and RBP accumulates in the liver. Injection of retinol (dispersed in a 20% Tween 40 solution) into deficient rats stimulated a rapid secretion of RBP from the liver into the serum. Colchicine treatment markedly inhibited the retinol-stimulated secretion of RBP from the liver into the serum. The effect of colchicine was most pronounced during the early period after retinol injection, particularly during the first 30 to 60 minutes. Ninety minutes after retinol injection, the serum RBP level of colchicine treated rats was only 36% as great as that of the control rats. In parallel experiments, a quantitatively similar inhibition of very low density lipoprotein (VLDL) secretion by colchicine was observed in the retinol-deficient rats. In contrast, colchicine did not affect the overall rate of hepatic protein synthesis, as estimated from the incorporation of [^{3}H]leucine into total liver and serum protein; the secretion of newly synthesized protein was, however, inhibited. When rats were treated with colchicine and then injected with retinol, the level of RBP in a Golgi-rich fraction obtained from the liver homogenate increased markedly as compared to the level of prealbumin. The inhibition of RBP secretion by colchicine suggests that the microtubules play a role in RBP secretion. By analogy to studies on VLDL and albumin, these data provide presumptive evidence that the Golgi apparatus and secretory vesicles are involved in RBP secretion.—**Smith, J. E., D. D. Deen, Jr., D. Sklan, and D. S. Goodman.** Colchicine inhibition of retinol-binding protein secretion by rat liver. *J. Lipid Res.* 1980. **21:** 229–237.

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Vitamin A normally circulates in plasma mainly as the alcohol, retinol, bound to a specific transport protein, retinol-binding protein (RBP). RBP is synthesized in the liver (1, 2) and is secreted into the plasma largely as the retinol-RBP complex (holo-RBP) (3, 4). Secretion of RBP by the liver is a highly regulated process. Previous studies in the rat have shown that vitamin A deficiency results in a specific block in the secretion of RBP by the liver, leading to a decrease in the plasma level of RBP and the concomitant several-fold increase of RBP (as apo-RBP) in the liver (5, 6). Upon vitamin A repletion, RBP is rapidly secreted from the expanded liver pool into the plasma as holo-RBP (7, 8).

The drug colchicine has been shown to inhibit the secretion of several plasma proteins that are produced in the liver (9–11). The inhibition appears to occur at a site between the formation of Golgi-derived secretory vesicles and their fusion with the plasma membrane (9, 12, 13), and may be related to the disruption of microtubules induced by colchicine (14).

We now report studies on the inhibition by colchicine of the retinol-stimulated secretion of RBP by the livers of retinol-deficient rats. These studies are part of a research program in our laboratory that aims to explore in detail the cellular and molecular mechanisms involved in the regulation of RBP secretion by the liver.

METHODS

Rats

Male weanling rats of the Holtzman strain were depleted of their liver vitamin A stores as described previously by feeding a vitamin A-deficient diet (5, 15) (approximately 40 days) until all of the rats had serum vitamin A levels of 3 μg/dl or less. The rats were then maintained on an identical diet supplemented with 12 mg of retinoic acid per kg of diet. The rats were housed in an air conditioned room at 22°C, in

Abbreviations: RBP, retinol-binding protein; VLDL, very low density lipoprotein; HDL, high density lipoprotein.

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stainless steel pan-type cages with wood chip bedding. Since these rats were not truly vitamin A deficient, we have called them "retinol-deficient" rats.

Preparation of solutions

Dispersions of retinol for injection into the rats were prepared by dissolving an appropriate amount of retinol in 0.1 ml of ethanol, followed by the addition of 4 ml of Tween 40 (Sigma, St. Louis, MO) (under N_2 at 40°C). With constant stirring, 8 ml of a solution of 0.9% NaCl and then 8 ml of water, were added one drop at a time. Thus, the final clear solution contained retinol in a solution of 20% (by volume) Tween 40 in 0.36% (w/v) NaCl. This solution will be referred to as the retinol carrier solution in this paper.

Colchicine (Sigma) was dissolved in 0.9% NaCl (Abbott Laboratories, North Chicago, IL) (2.5 g/l). This solution was prepared fresh on the day of the experiment.

Assays

Liver and serum samples were assayed for vitamin A by the fluorometric-correction formula methods of Thompson et al. (16), using precautions previously described (5).

RBP was determined by a modification of our previously described radioimmunoassay (2, 5, 15). The pure rat serum RBP used in the assay was iodinated by the lactoperoxidase procedure of Miyachi et al. (17), instead of by the chloramine-T method. All liver homogenates were treated with 1% Triton X-100 before dilution, in order to ensure that all RBP would be accessible to antibody in the immunoassay, instead of using the extensive homogenization procedure previously described (2, 5, 15). Triton X-100 added at levels 10-fold greater than the amounts used in these experiments had no other effect on the results of the radioimmunoassay.

Prealbumin was determined on the Triton X-100 treated samples by the previously described radioimmunoassay (8).

Previous studies have shown that, in both the RBP and prealbumin assays, the radioactive protein displacement curves obtained with the purified proteins, serum, and liver homogenates are all identical (5, 8). At present, it is not known if there are precursors of RBP or prealbumin in liver which cross-react with these antibodies.

Protein was estimated by the dye-binding method of Bradford (18) using bovine serum albumin as a standard.

Galactosyltransferase activity was determined by measuring the transfer of [^{14}C]galactose from UDP-[^{14}C]galactose (283 Ci/mol) (New England Nuclear, Boston, MA) to acid-hydrolyzed bovine submaxillary mucin. The assay was conducted in a manner similar to the assay described by Grimes (19).

Extraction of ^{14}C-labeled lipid from serum

Samples of 0.5 ml of serum were mixed with 10 ml of chloroform–methanol 2:1 (v/v). The mixture was separated into two phases with 2.5 ml of 0.05% H_2SO_4. The chloroform phase was removed and dried under a stream of nitrogen. The residue was dissolved in 15 ml of toluene containing 0.5% diphenyloxazole and assayed for ^{14}C in a Packard model 3365 liquid scintillation counter.

Isolation and counting of ^{3}H-labeled protein

Protein was quantitatively isolated from samples of 0.5 g of liver and 0.5 ml of serum by the method of Siekevitz (20). The isolated protein was dissolved in 1 ml of NCS solubilizer (Searle Analytical, Chicago, IL). Ten ml of Aquasol (New England Nuclear) was added and was assayed for radioactivity in the above scintillation counter.

Experimental protocols

Four studies were conducted to explore the effects of colchicine on the retinol-stimulated secretion of RBP by the liver and on other related phenomena. In an initial preliminary experiment (Study #1), retinol-deficient rats each received two intraperitoneal (i.p.) injections of colchicine (5 mg/kg body weight, each time) at 100 and at 10 min before the administration of retinol. Retinol was injected intravenously (i.v.) at time 0, and the rats were killed 90 min later. Serum and liver samples were collected and assayed for vitamin A and RBP levels, and the results were compared with those in control rats injected with 0.9% NaCl instead of colchicine.

Three more extensive experiments were then conducted. Since Stein, Sanger, and Stein (13) reported that a single intraperitoneal injection of 5 mg of colchicine per kg body weight given 3 hr prior to the injection of tracer resulted in a maximum inhibition of very low density lipoprotein (VLDL) secretion, this time interval was selected for our subsequent experiments.

A major experiment (Study #2) was conducted to compare the effects of colchicine on the secretion of RBP, VLDL, and other newly synthesized proteins in retinol-deficient rats. The retinol-deficient rats were given an i.p. injection of either 2 ml of 0.9% NaCl/kg body weight (20 rats) or 5 mg of colchicine/kg body weight (16 rats). After 3 hr, the rats were divided among the three parts of the study which were con-

ducted simultaneously. The rats assigned to the RBP part of the study were injected i.v. with either 0.5 ml of the retinol carrier solution containing 50 μg of retinol, or 0.5 ml of the carrier solution alone, via the tail vein. The rats assigned to the part of the study dealing with the secretion of VLDL were injected i.v. with 1 ml of a solution which contained 0.1 g Triton WR 1339, 3.33 μCi of sodium [1-^{14}C]palmitate (54.3 Ci/mol) (New England Nuclear) and 4 mg of bovine serum albumin in 0.9% NaCl via the tail vein. The remaining rats were used to study the overall effects of colchicine on the synthesis and secretion of proteins by the liver. These rats were given an injection into the tail vein of 10 μCi of L-leucine (4,5-^{3}H) (50 μCi/mmol) (New England Nuclear) in 1 ml of 0.9% NaCl. All rats were killed by decapitation 90 min after the second (the i.v.) injection. Serum and liver samples were collected for the appropriate analyses.

Another major experiment (Study #3) was conducted to explore the effects of colchicine upon the amount and level of RBP localized in the Golgi apparatus. Twenty retinol-deficient rats were evenly divided among four treatments: *1*) saline-Tween 40, *2*) colchicine-Tween 40, *3*) saline-retinol, and *4*) colchicine-retinol. Due to the time required to prepare the Golgi-rich fractions, only three rats were killed on any day, and rats from three separate treatments were used. The rats were given an i.p. injection of either 5 mg of colchicine per kg body weight or 2 ml of 0.9% NaCl per kg body weight. Three hours later, the rats were anesthetized and either 1 ml of the Tween 40 carrier solution containing 600 μg of DL-α-tocopherol or 1 ml of the Tween 40 solution containing 150 μg of retinol and 600 μg of DL-α-tocopherol was administered i.v. through the dorsal vein of the penis. The rats were decapitated 4.5 hr after the initial (i.p.) injection. Serum and liver samples were collected. The liver samples were divided into two portions, one for vitamin A analysis and the other for the preparation of the Golgi-rich fractions by the method of Morré (21). The marker enzyme galactosyltransferase was used to quantify the recovery of the Golgi. RBP was measured in the liver homogenates used to prepare the Golgi-rich fractions as well as in the Golgi-rich fractions.

A study (Study #4) on the time-course of the colchicine inhibition of the retinol-stimulated secretion of RBP was also conducted. Details of this experiment are provided in the Results section.

Statistical treatment of data

All data are reported as means ± the standard error of the mean. Statistical comparisons were made by either Student's *t*-test or one-way analysis of variance (22). When significant differences were detected by analysis of variance, the appropriate comparisons were made by Tukey's ω-procedure (22).

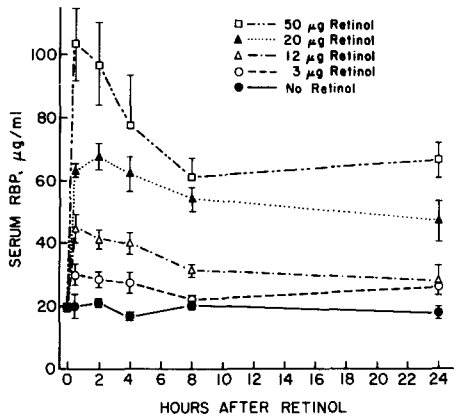

Fig. 1. Serum RBP concentrations of retinol-deficient rats injected with 20% Tween 40 carrier solution containing varying amounts of retinol. Each rat was injected with 1 ml of the 20% Tween 40 carrier solution containing the indicated amount of retinol. Blood samples of approximately 0.7 ml were drawn by the venipuncture technique of Phillips, Stafford, and Stuut. (34). Each data point represents the mean ± SEM of three samples.

RESULTS

Effect of retinol administered in a Tween 40 carrier solution on the secretion of RBP by the liver

In our previous studies, vitamin A was injected into deficient rats as newly absorbed vitamin A in chylomicrons (7). This was done because vitamin A normally reaches the liver in the form of retinyl esters in association with chylomicron remnants (23, 24). This form of administration of vitamin A was felt to be inadvisable in the present studies with colchicine, however, because colchicine has been reported to interfere with the normal uptake of chylomicrons (25), and with the hydrolysis of chylomicron cholesteryl esters (26) by hepatocytes.

A study was designed to determine if retinol dispersed in a 20% Tween 40 solution would effectively stimulate the secretion of RBP from the livers of retinol-deficient rats. The data shown in **Fig. 1** indicate that the i.v. injection of retinol administered in the Tween 40 carrier solution produced a rapid, dose-related increase in the serum concentration of RBP. The rise in serum RBP levels presumably reflected the retinol-stimulated secretion of RBP from the liver into the blood. The level of RBP in serum at 30 min and 2 hr after the injection of retinol was highly correlated with the amount of retinol injected (correlation coefficients 0.95 and 0.93, respectively). The changes in serum vitamin A concentration in response to the injection of retinol in Tween 40 were very similar (in molar terms) to the changes seen in the levels of serum RBP (data not shown).

TABLE 1. Effect of colchicine and retinol on the serum and liver concentrations of RBP and vitamin A

Colchicine	Retinol[a]	No. of Rats	Serum Vitamin A	Serum RBP	Liver Vitamin A	Liver RBP
mg/kg BW[a]	*μg*		*μg/dl*	*μg/ml*	*μg/liver*	*μg/g*
None[b]	None[c]	5	1.9 ± 0.5[d]	9.2 ± 2.6[d]	0.9 ± 0.5[d]	153 ± 19[e]
5	None[c]	5	2.0 ± 0.5[d]	6.2 ± 2.1[d]	0.9 ± 0.5[d]	137 ± 16[e]
None[b]	150	4	52.4 ± 4.2[f]	72.3 ± 4.2[f]	64.9 ± 2.5[e]	47 ± 2[d]
5	150	4	20.8 ± 2.4[e]	35.2 ± 9.6[e]	68.8 ± 4.5[e]	143 ± 16[e]

[a] Retinol was injected 3 hr after the initial injection. The rats were killed 90 min later. The mean body weight (BW) of the rats used in this experiment was 372 ± 9 g.
[b] Rats were injected with 2 ml of a 0.9% NaCl solution per kg body weight.
[c] Rats were injected with 1 ml of the 20% Tween 40 carrier solution containing 600 μg of DL-α-tocopherol.
[d,e,f] Means ± SEM; values with different superscripts are significantly different, $P < 0.05$.

The changes in serum RBP levels seen after the injection of retinol in a 20% Tween 40 solution closely resembled those previously seen after the injection of vitamin A in association with lymph chylomicrons (7). However, the amount of retinol required to stimulate the secretion of a given amount of RBP from the liver (i.e., to obtain a given serum level) was about 2- to 3-times that required in our previous study (7), when vitamin A was injected in chylomicrons. This quantitative difference is probably due to differences in the tissue distribution pattern of vitamin A when injected in the Tween 40 solution, compared to its administration in the form of chylomicrons. Most of the chylomicron vitamin A is removed from the circulation by the liver (23). In contrast, much of the vitamin A would be expected to be taken up by the reticulo-endothelial cells of the spleen, liver, and lungs when vitamin A is administered in an artificial form (27).

TABLE 2. Effect of colchicine on the retinol-stimulated release of RBP[a]

Colchicine	Retinol[b]	No. of Rats	Serum RBP	Increase in Serum RBP	Relative Change
mg/kg BW	*μg*		*μg/ml*	*μg/ml*	*%*
None[c]	None[d]	5	5.6 ± 0.4[e]		
None[c]	50	3	52.6 ± 4.8[f]	47.0	100
5	50	6	22.7 ± 2.5[g]	17.1	36

[a] This experiment was conducted simultaneously with the experiments reported in Tables 3 and 4 (Study #2, see Methods section).
[b] Retinol was injected 3 hr after the initial injection. The rats were killed 90 min after the retinol injection.
[c] Rats were injected with 2 ml of a 0.9% NaCl solution per kg body weight.
[d] Rats were injected with 0.5 ml of the 20% Tween 40 carrier solution.
[e,f,g] Means ± SEM; values with different superscripts are significantly different, $P < 0.01$.

Effects of colchicine on the retinol-stimulated secretion of RBP by the liver

Effects on serum RPB levels. In each of four separate experiments in which different amounts of retinol were injected into retinol-deficient rats, prior treatment with colchicine dramatically decreased the extent of the subsequently observed rise in serum levels of RBP. In our initial experiment (Study #1), only 20 μg of retinol was injected. The serum RBP level of the control group increased an average of 21 μg/ml during the subsequent 90 min, whereas the rats pretreated with colchicine showed an average rise of only 13 μg/ml. The results of more extensive experiments, in which larger amounts of retinol were injected, are presented in **Tables 1** and **2.** As shown in Table 1, when retinol-deficient rats were injected with 150 μg of retinol, serum RBP levels increased 90 min later from an average value of 9.2 μg/ml to 72.3 μg/ml. In rats pretreated with colchicine, however, the serum RBP levels rose to a mean of only 35.2 μg/ml. Similarly, injection of 50 μg of retinol to control rats resulted in an average increase in serum RBP of 47 μg/ml (Table 2); pretreatment with colchicine reduced this rise to only 17.1 μg/ml (36% as great as that seen with the control rats). Furthermore, in all of the experiments, the relative changes in the serum vitamin A levels closely reflected the changes observed in the serum RBP levels (see Table 1).

The time-course of the effects of colchicine on the retinol-stimulated secretion of RBP is shown in **Fig. 2.** Thirty min after the injection of retinol, the serum RBP levels of colchicine-treated rats increased only 20% as much as did those of control rats. The serum RBP levels of the control (saline treated) rats reached a maximum level by approximately 50 min, and remained at this level for the remainder of the

experiment (to 120 min). In contrast, the serum RBP levels of the colchicine-treated rats continue to increase slowly in a linear fashion throughout the entire duration of the experiments, thus reducing the magnitude of the difference between the two groups at later times.

Effects on liver RBP levels. Administration of retinol to retinol-deficient rats that did not receive colchicine resulted in a marked decrease in liver RBP levels concomitant with the increase in serum RBP (Table 1, line 3), similar to results reported previously (7). In contrast, rats injected with colchicine and then given retinol had liver RBP levels which were similar to those of the rats which received neither colchicine nor retinol (Table 1, lines 1 and 4). Similar results were consistently observed in all three experiments where liver RBP levels were determined. These results were rather surprising, since the rats treated with both colchicine and retinol showed significant, albeit lesser, increases in the serum levels of RBP. Thus, in the experiment shown in Table 1, if the entire rise of serum RBP came from a preformed liver pool, and no new RBP synthesis took place in the liver, the expected liver RBP levels of the rats treated with both colchicine and retinol would have been approximately 90 μg/g (instead of the observed 143 μg/g). The results suggest that RBP synthesis was stimulated after retinol injection, and that the increase in liver RBP in the rats also treated with colchicine roughly offsets the amount of RBP secreted from the preformed liver pool (see also Discussion section). Another consistent finding was that the administration of colchicine alone did not increase the level of liver RBP found in retinol-deficient rats (see Table 1).

The data shown in Tables 1 and 2 and in Fig. 2 indicate that colchicine treatment inhibited the retinol-stimulated secretion of RBP by the liver. The possibility was also considered, however, that the effects of colchicine might have been due in part to an alteration in the amount of retinol taken up by the hepatocytes (leading to a reduced stimulus for RBP secretion). Accordingly, an experiment was conducted with rats with large liver stores of vitamin A (2 mg/liver), fed a commercial rat chow. The large stores of vitamin A in these rats actively promoted the secretion of RBP by the liver, and these rats had liver RBP levels typical of normal rats (21 ± 2 μg/g). Treatment of these chow-fed rats with colchicine (5 mg/kg body weight) resulted in an increase in liver RBP levels to 48 ± 3 μg/g during the subsequent 4.5 hr. This experiment further demonstrated that colchicine administration led to a block in RBP release by the liver.

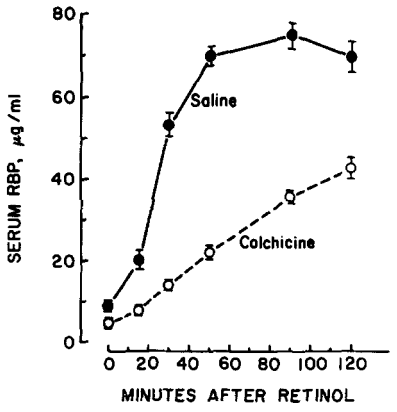

Fig. 2. The effects of colchicine on the serum RBP response of retinol-deficient rats to the injection of 150 μg of retinol in 20% Tween 40 carrier solution (Study #4, see Methods section). The rats were injected with either 5 mg of colchicine in 2 ml of 0.9% NaCl per kg bodyweight, or with 0.9% NaCl alone, 3 hr prior to the retinol injection. Each rat was then injected with 1 ml of the 20% Tween 40 carrier solution containing 150 μg of retinol and 600 μg of DL-α-tocopherol through the dorsal vein of the penis while under light ether anesthesia. Blood samples of approximately 0.7 ml were drawn by the venipuncture technique of Phillips et al. (34). Each data point shown represents the mean ± SEM for eight rats. The mean weight of the rats used in this experiment was 408 g.

Effects of colchicine on the secretion of VLDL and on protein synthesis by the liver

An experiment was conducted to compare the inhibition by colchicine of the retinol-stimulated secretion of RBP with the cholchicine inhibition of VLDL secretion in retinol-deficient rats. In this experiment (Study #2, see Methods section), the retinol-stimulated increase in serum RBP level in the colchicine treated group was only 36% of that observed in the control (saline treated) rats (see Table 2).

The effects of colchicine on the secretion of VLDL were explored by the methods employed by Stein et al. (13) for the purpose. The experiments were conducted as described by Stein et al. (13), except that we used retinol-deficient rats. The rats were injected with Triton WR 1339 in order to block removal of VLDL from the plasma compartment. More than 95% of the ^{14}C-labeled lipid that accumulates in serum under these conditions (after [^{14}C]palmitate injection), has been shown to be in the form of triglycerides (10, 13). The data presented in **Table 3** demonstrate that the colchicine administered in these studies markedly inhibited the secretion of labeled triglyceride (presumably reflecting VLDL secretion) into the plasma. The results (Table 3) are in good agreement with those previously reported by Stein et al. (13). The degree of inhibition of ^{14}C-labeled lipid secretion by colchicine (Table 3) was quantitatively quite similar

TABLE 3. Effect of colchicine on the release of ^{14}C-labeled lipid in rats injected with Triton WR 1339

Colchicine[a]	No. of Rats	^{14}C-labeled Lipid in Serum	Relative Change
mg/kg BW		*cpm/ml* × 10^{-3}	*%*
None	4	34.3 ± 1.9	100
5	5	12.8 ± 1.1[b]	37

[a] Three hr after the injection of colchicine (or saline), each rat was injected with Triton WR 1339 and [1-^{14}C]palmitate (see Methods section, Study #2). The rats were killed 90 min after the second injection.
[b] Significantly different from the other treatment, $P < 0.005$.

to the degree of inhibition of the retinol-stimulated secretion of RBP (Table 2).

In the third part of this study, the effects of colchicine on the incorporation of [^3H]leucine into liver and serum proteins were explored. This experiment was also conducted in a manner similar to the study of Stein et al. (13), except that retinol-deficient rats were employed. As shown in **Table 4,** colchicine did not inhibit protein synthesis, since it did not affect the incorporation of [^3H]leucine into total liver and serum protein in retinol-deficient rats. Colchicine did, however, block the secretion of newly-synthesized protein, since the proportion of the labeled protein found in the serum was markedly decreased in the colchicine treated as compared to control rats.

Effects of colchicine on the level of RBP in rat liver Golgi-rich fractions

Electron microscopy studies have shown that colchicine administration results in the accumulation of VLDL in Golgi-derived secretory vesicles (13). In addition, colchicine has been shown to cause albumin to accumulate in fractions of Golgi cisternae and Golgi-derived secretory vesicles (9, 12). A study (Study #3, see Methods section) was conducted to investigate whether colchicine would have similar effects upon the localization of RBP in the Golgi apparatus, after RBP secretion was stimulated by injection of retinol. For comparison, the effects of the various treatments upon the levels of prealbumin in the Golgi apparatus were also determined.

The results of this study are shown in **Table 5.** Colchicine-treatment alone led to a small and statistically not significant increase in the Golgi level of RBP. This small effect of colchicine alone probably reflects the greatly reduced rate of RBP secretion present in retinol-deficient rats. In contrast, colchicine considerably and significantly increased the Golgi level of RBP in the rats in which RBP secretion was stimulated by injection of retinol.

Treatment with either colchicine alone, or with colchicine and then retinol, increased the level of prealbumin in the Golgi-rich fractions. The effects of the two treatments were quantitatively similar. These results are consistent with our previous findings that, unlike with RBP, the rate of secretion of prealbumin by the liver is neither inhibited by retinol deficiency, nor stimulated by injection of retinol into retinol-depleted animals (8).

The marked increase in the RBP level of the Golgi-rich fractions of the rats treated with both colchicine and retinol was associated with a significant increase (to approximately 2-fold) in the molar ratio of RBP to prealbumin in these fractions. These data thus demonstrate that colchicine resulted in the accumulation of RBP in the Golgi-rich fraction, after RBP secretion was stimulated by the injection of retinol.

DISCUSSION

These studies were designed to examine in detail the effects of colchicine on the secretion and metabolism of RBP by the liver. Previous studies have shown that RBP secretion is strongly influenced by the vitamin A status of the animal. In the vitamin A deficient rat, the rate of secretion of RBP from the liver into the serum is greatly reduced, and RBP accumulates in the liver (5). Administration of vitamin A to such a deficient rat leads, in turn, to the rapid secretion of RBP from the expanded liver pool into the serum (5, 7). Since RBP secretion can be specifically inhibited or stimulated by retinol depletion and repletion, respectively, the retinol-deficient rat provides an excellent model for the study of other agents which may inhibit or stimulate the secretion of RBP.

The rats used in these studies were first depleted of their vitamin A stores, and were then maintained on the same diet supplemented with retinoic acid. Retinoic acid is transported in plasma bound to serum albumin and not to RBP (28), and has no detectable effect on RBP metabolism in vitamin A deficient rats (5). We have designated these retinoic acid-fed rats

TABLE 4. Effect of colchicine on the incorporation of ^3H-labeled leucine into protein in liver and serum

Colchicine[a]	No. of Rats	Total ^3H-labeled Protein of Liver + Serum[b]	^3H-labeled Protein in Serum
mg/kg BW		*cpm* × 10^{-3}	*%*
None	4	103.0 ± 4.7	39.8 ± 1.2
5	5	104.7 ± 5.1	20.2 ± 1.9[c]

[a] Three hr after the colchicine (or saline) injection, the rats were injected with L-[4,5-^3H]leucine (see Methods section, Study #2).
[b] The serum volume was assumed to be 3.5% of the body weight.
[c] Significantly different from other treatment, $P < 0.005$.

TABLE 5. Effect of colchicine and retinol on the concentration of RBP and prealbumin in liver Golgi-rich fractions[a]

Colchicine	Retinol[b]	No. of Rats	RBP in Golgi-rich Fraction		Prealbumin in Golgi-rich Fraction		RBP/Prealbumin Molar Ratio
mg/kg BW	*μg*		*μg/mg protein*	*μg/unit transferase*[c]	*μg/mg protein*	*μg/unit transferase*[c]	
None	None	5	0.20 ± 0.03[d]	0.81 ± 0.21[d]	0.15 ± 0.01[d]	0.58 ± 0.12[d,e]	3.56 ± 0.41[d]
5	None	5	0.27 ± 0.03[d]	1.63 ± 0.25[d]	0.29 ± 0.02[e]	1.82 ± 0.34[f]	2.52 ± 0.33[d]
None	150	4	0.13 ± 0.01[d]	0.34 ± 0.03[d]	0.15 ± 0.02[d]	0.36 ± 0.07[d]	2.62 ± 0.37[d]
5	150	4	0.47 ± 0.09[e]	3.01 ± 0.35[e]	0.20 ± 0.03[d]	1.39 ± 0.28[e,f]	6.27 ± 1.21[e]

[a] Additional data from the rats in this experiment are shown in Table 1.
[b] Retinol was injected 3 hr after the initial injection, and the rats were killed 90 min later.
[c] Galactosyltransferase activity was expressed as nmol of galactose transferred to the acceptor protein in 20 min, with 1 unit = 1 nmol transferred.
[d,e,f] Means ± SEM; values with different superscripts are significantly different, $P < 0.05$.

as "retinol-deficient" to differentiate them from the classical vitamin A-deficient rat which does not grow and is frequently in poor health.

In every one of the experiments that we conducted, colchicine markedly inhibited the retinol-stimulated secretion of RBP from the liver into the serum. The effect of colchicine was most pronounced during the early period after retinol injection (particularly during the first 30 to 60 min, see Fig. 2), and was of lesser magnitude at later times. These results (Fig. 2) suggest that a major effect of colchicine was to retard, rather than to fully block, the retinol-stimulated secretion of RBP. A similar conclusion, namely that the effect of colchicine may be "best defined as a retardation rather than mere inhibition", was recently presented by Malaisse-Lagae et al. (29), in studies on the effects of colchicine on the secretion of insulin by the pancreatic B cell.

Since retinol-deficient rats do have some metabolic abnormalities (30, 31), a study was conducted to determine if these rats responded to colchicine treatment in the same manner as reported for normal rats fed a commercial rat chow. Colchicine has been shown to inhibit the secretion of several serum proteins produced by the liver, including albumin (9, 12), fibrinogen (11), and the lipoproteins VLDL (10, 13, 14) and HDL (13). In the present study, the inhibitory effect of colchicine on the secretion of RBP was compared with its effect on VLDL secretion. The inhibition of VLDL secretion by colchicine observed in the retinol-deficient rats was very similar to that previously reported for normal rats (10, 13). In fact, the effects of colchicine on the relative changes in the serum levels of RBP and of VLDL were quantitatively comparable, suggesting that the inhibition of secretion of these two proteins may involve a similar mechanism.

In contrast to its inhibitory effects on the secretion of RBP and VLDL by the liver, colchicine did not affect the overall rate of hepatic protein synthesis, as estimated from the extent of incorporation of ^{3}H-labeled leucine into total liver and serum protein. The relative amount of the labeled protein found in serum, as compared to liver, was, however, significantly decreased by colchicine. Again, these results with retinol-deficient rats were similar to those previously reported for normal rats (13).

When the levels of RBP in the livers of the colchicine-treated rats were examined, it was found that these levels remained high whether the rats were injected with retinol or not. In fact, the liver RBP levels of rats treated with colchicine alone, or with colchicine and then retinol, were indistinguishable from those of the untreated retinol-deficient rats. In contrast, administration of retinol alone to the retinol-deficient rats resulted in a rapid decrease in the level of RBP in the liver, concomitant with the rapid rise in the level of RBP in the serum. Previous studies have shown that the increment in RBP found in serum immediately after retinol injection mainly represents RBP secreted from a preformed pool in the liver. Accordingly, since significant (albeit reduced) amounts of RBP were secreted into the serum in colchicine treated rats after retinol injection, we were surprised to find that the liver RBP levels were not reduced in these animals. It was evident that the rats treated with both colchicine and retinol had a larger total body pool of RBP than did the rats which received neither of these treatments, or colchicine alone. The additional RBP found in the rats treated with both colchicine and retinol presumably arose by de novo synthesis of RBP which occurred during the experiment. Previous studies, both with intact rats (7) and with rat liver cells in culture in vitro (2), have suggested that the provision of retinol to a retinol-deficient liver cell first stimulates RBP secretion by the cell, and then secondarily stimulates RBP synthesis. We suggest that, in the presence of colchicine, where RBP secretion was inhibited, administration of retinol still stimulated RBP synthesis, resulting in the maintenance of a high

level of RBP in the liver cell and an enlarged total body pool of RBP in the rat.

Studies on the subcellular distribution of RBP in liver have shown that most of the RBP is found associated with the microsomal fraction, both in normal and in vitamin A deficient rats (15, 32). Additional studies have shown that both the smooth, and particularly the rough, microsome fractions are enriched with RBP[3]. Analyses of Golgi-rich fractions demonstrated that, although the Golgi contained significant amounts of RBP, the Golgi apparatus was not quantitatively the major site of localization of RBP in the liver cell (15). More recent studies, employing several glycosyltransferases as Golgi marker enzymes, have shown the Golgi apparatus to contain a maximum of 23% of the liver RBP in the normal rat, and a maximum of 9% of the liver RBP in the retinol-deficient rat (33).

Colchicine has been shown to cause VLDL and albumin to accumulate in Golgi and Golgi-derived secretory vesicles (9, 12, 13). In the present study, a similar phenomenon was observed with RBP. Thus, when rats were first treated with colchicine and then injected with retinol (to stimulate RBP secretion), the level of RBP in the Golgi-rich fraction increased markedly as compared to the level of prealbumin. Calculations based on the assumptions that all of the liver galactosyltransferase activity was associated with the Golgi apparatus, and that all of the RBP in the Golgi-rich fraction was associated with the Golgi apparatus, indicate that 34 ± 6% of the liver RBP was found in the Golgi in the colchicine- and retinol-treated rats. These data strongly suggest that the Golgi apparatus is involved in the secretion of RBP. The data are also consistent with the idea that the site of the inhibition of RBP secretion caused by retinol deficiency is located in cells somewhere between the site of RBP synthesis in the endoplasmic reticulum and the Golgi apparatus.

It is worth noting that the Golgi level of RBP was substantially increased in the rats treated with both colchicine and retinol, while as discussed above the total liver level of RBP of these rats was not significantly different from that of the retinol deficient rats which had not received either treatment. Thus, treatment with both colchicine and retinol appears to significantly alter the subcellular distribution of liver RBP. This suggests that the level of RBP in a fraction or fractions of liver other than the Golgi apparatus was markedly lowered, although direct data dealing with this subject were not obtained.

[3] Smith, J. E., J. A. Resnick, and D. S. Goodman. Unpublished observations.

While the exact mechanism by which colchicine blocks protein secretion remains to be fully defined, the effects of colchicine presumably reflect in large part the colchicine-induced disruption of the microtubular system within the liver cell. In a recent study of the pancreatic B cell it was shown that colchicine causes a time-related decrease in the number of microtubules that was correlated with its effects on insulin secretion (29). Studies on VLDL secretion have traced the VLDL molecules from their site of synthesis in the endoplasmic reticulum, through the Golgi apparatus and secretory vesicles to the sinusoidal cell surface (13). Treatment with colchicine led to an apparent reduction in microtubules and the accumulation of Golgi-derived vesicles containing nascent VLDL particles (13, 14). It was suggested that intact microtubules might be required for the operation of the final steps of the VLDL secretory cycle. The quantitatively similar inhibition of RBP and VLDL secretion by colchicine reported here suggests that a similar secretory process is involved in the hepatic secretion of both RBP and VLDL. Thus, by analogy to VLDL and albumin, the inhibition of RBP secretion by colchicine and the accumulation of RBP in Golgi-rich fractions after colchicine administration provide presumptive evidence that the Golgi apparatus and secretory vesicles are involved in the process of RBP secretion.

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