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

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ABSTRACT

Rats were injected with colchicine and the secretion of triglycerides into the serum was studied for 90 min after injection of [^{14}C]palmitic acid and Triton WR 1339. The release of labeled and chemically determined triglyceride was reduced to about 20–30% of control values. The effect of colchicine on serum triglyceride levels was not dependent on the presence of Triton and was similar in males and females and in fed and fasted rats. The effect was dose dependent and was reversible 6–7 h after injection of 0.05 mg/100 g body weight. Colchicine inhibited also the release of labeled proteins into the serum but did not affect the amount of [^{3}H]leucine incorporated into liver proteins. Within 4 h of colchicine treatment there was an 80% fall in serum very low density lipoproteins (VLDL), a 30% fall in serum high density lipoproteins (HDL), and no change in the $d > 1.21$ protein level, but reduction in the appearance of labeled proteins was encountered in all serum fractions. Colchicine had no effect on the rate of bile flow and on the secretion of phospholipids and cholesterol into the bile. In the hepatocyte there was accumulation of Golgi-derived secretory vesicles, containing nascent VLDL particles; these vesicles were seen also in the vicinity of the sinusoidal cell surface, but the space of Disse contained few or no VLDL particles. There was an apparent reduction in microtubules and some increase in microfilaments. It is suggested that microtubules affect the secretion of lipoproteins and proteins into the serum by maintaining the organization of the plasma membrane required for its fusion with secretory vesicles. The lack of effect of colchicine on biliary lipid secretion indicates that the latter is not dependent on vesicular transport.

INTRODUCTION

The mammalian liver cell secretes lipids into the blood stream in the form of lipoproteins and into the bile in the form of lipid micelles. The intracellular pathways leading to the secretion of lipo-

proteins into the blood stream have been studied extensively (1–3); however, not much is known about the regulation of the final steps, i.e. the transport of the secretory vesicles from the Golgi apparatus towards the sinusoidal cell surface and the release of the secretory products. Recently, we have examined the effect of agents known to affect the microtubular system on the release of very low density lipoproteins (VLDL) into the circulation and have shown that, in the rat, colchicine interferes with the secretion of these lipoproteins (4). Colchicine did not affect the synthesis of triglycerides by the liver, nor did it cause inhibition of protein synthesis (4). These findings were further corroborated by others (5, 6) in the perfused mouse liver. Presently, information was sought whether the secretion of high density lipoproteins (HDL) and of proteins into the serum as well as of phospholipids and cholesterol into the bile is also affected by colchicine administration. An attempt was made also to define more closely the optimal conditions for studying the effects of colchicine on the secretory activity of rat liver in the intact animal.

MATERIALS AND METHODS

Animals and Experimental Procedure

Female and male 200 g albino rats of the Hebrew University strain, fed the pelleted diet Am Rod 931 (7) and kept in constant temperature room, were used. In some experiments the rats were given 10% sucrose in drinking water for 5 days in order to raise serum VLDL levels. Colchicine (Sigma Chemical Co., St. Louis, Mo.) was dissolved in 0.9% NaCl immediately before use and was injected intraperitoneally in a single dose of 0.05 or 0.5 mg/100 g body weight. The control rats were given 0.9% NaCl. To study the rate of secretion of liver triglycerides into the circulation, all rats received 1 μCi of [1-^{14}C]palmitic acid (specific activity 59 Ci/mol) prepared as a sodium salt complexed to bovine albumin (8) followed by 0.5 ml of 20% solution of Triton WR 1339 (Serva, Heidelberg, Germany) in 0.9% NaCl, injected into an exposed femoral vein. The rats were killed by exsanguination 90 min thereafter. In order to study the protein moiety of serum lipoproteins, female rats were injected intraperitoneally with colchicine (0.5 mg/100 g body weight) and 180 min later were injected intravenously with 10–40 μCi of L-[4,5-^{3}H]leucine (specific activity 36 Ci/mmol). The rats were killed by exsanguination 60 min after injection of leucine. Liver and serum proteins were isolated and purified according to Siekevitz (9).

Vinblastine sulfate (Eli Lilly and Co., Indianapolis, Ind.) 0.1 or 1.0 mg/100 g body weight was injected intraperitoneally and was followed at different time intervals by intravenous injections of [1-^{14}C]palmitic acid and Triton WR 1339. The rats were killed 90 min after injection of the labeled fatty acid.

In Vitro Study

Liver slices, prepared with the help of a Stadie Riggs slicer, were incubated in Krebs-Ringer bicarbonate buffer containing 0.5% bovine serum albumin and 10 mM glucose at 38°C in 95% O_2-5% CO_2 for 3 h. Vincristine sulfate (Eli Lilly and Co.) or vinblastine sulfate was added to the incubation medium to give a final concentration of 10^{-5} or 10^{-6} M.

Isolation of Lipoprotein Fractions

In several experiments the sera of experimental and control rats were pooled and lipoproteins were isolated by ultracentrifugation according to Havel et al. (10) in a Spinco model L265B ultracentrifuge (Beckman Instruments, Inc., Spinco Div., Palo Alto, Calif.).

VLDL were isolated at density 1.006 using a 50.1 rotor at 192,000 *g* for 16 h. After isolation by the tube slicing technique the VLDL were washed twice at density of 1.006, using a SW41 rotor for 16 h at 201,000 *g*. After removal of VLDL the serum was adjusted to density 1.085 with solid KBr and centrifuged in Ti 50 rotor at 150,000 *g* for 24 h. After removal of the $d < 1.085$ fraction the serum was adjusted to density 1.21 and centrifuged in SW41 rotor for 48 h at 201,000 *g*. The $d < 1.21$ fraction was isolated by tube slicing and refloated at d 1.21 for 48 h at 201,000 *g*; this fraction consisted of HDL, which proved to be of uniform size on negative staining.

Chemical and Radiochemical Procedure

Serum and liver lipids were extracted and washed according to Folch et al. (11). Samples of the purified chloroform extracts were taken for the determination of radioactivity and for separation into lipid classes by thin-layer chromatography, using Silica Gel-G plates and a solvent system consisting of petroleum ether (bp 30–60°C)-diethylether-glacial acetic acid (80:20:1, vol/vol/vol). The fractions, visualized by iodine vapor, were identified with the help of reference standards and the lipid classes were scraped into counting vials and assayed in a β-scintillation spectrometer (1). Serum triglycerides were determined by a semiautomated procedure (12). 5–10 mg of the purified liver and serum protein was dissolved in 0.5 ml Soluene (Packard Instrument Co., Inc., Downers Grove, Ill.) and the radioactivity was determined using the scintillation system consisting of toluene-dioxane-ethanol (5:3:2, vol/vol/vol) and 0.4% 2,5-diphenyloxazole and 0.015% dimethyl-1,4-bis-2-(4-methyl-5-phenyloxazolyl)benzol. All radioactivity measurements were performed with the Tri-Carb liquid scintillation spectrometer 3380, equipped with the absolute activity analyzer, model 544.

Preparation of Tissue for Electron Microscopy

Liver was fixed in either 4% osmium tetroxide for 2 h at 0°C or in half-strength Karnovsky's formaldehyde (prepared from paraformaldehyde) glutaraldehyde fixative (13) for 1–2 h followed by postfixation with 2% osmium tetroxide in acetate-Veronal buffer (14). The tissue was then subjected to either partial or complete dehydration (1) and embedded in Epon (15). Sections were prepared with the LKB ultratome using glass knives, were stained with uranyl acetate and lead citrate (16), and examined with the Philips EM 300 electron microscope at 60 kV.

Cannulation of the Bile Duct

Female rats weighing 220–250 g were used. Under ether anesthesia a Pe 10 polyethylene tube was tied into the proximal third of the bile duct, threaded through a subcutaneous tunnel, and exteriorized in the interscapular region (17). The rats were kept in restraining cages and had access to food and water. After the first 2 h of bile collection, the rats were injected into the tail vein with colchicine 0.2 mg/100 g body weight or with 0.9% NaCl. Collection of bile continued for two additional periods of 2 h each. Aliquots of bile were extracted with chloroform-methanol 2:1, and lipid phosphorus (18) and cholesterol (19) were determined on the chloroform extract.

RESULTS

The present experimental design, in which the removal of serum triglyceride was prevented by the administration of Triton WR 1339 (20), permitted us to study the rate of secretion of lipoproteins in the intact rat. The effect of a high and a low dose of colchicine on the release of labeled triglycerides as well as on the level of chemically determined triglycerides in the serum was studied in male and female rats (Table I), and the degree of inhibition in both sexes was found not to differ significantly. However, the rate of triglyceride secretion in the control rats was higher in the female, and so the next series of experiments was performed on female rats. In non-Triton-treated rats, injection of colchicine resulted in a fall of serum triglyceride levels of the same magnitude observed in rats given Triton (Table II). The duration of effect of colchicine was related to the dose used (Table III), and with the low dose the maximal reduction was observed after 270 min. With 0.05 mg/100 g body

TABLE II

Effect of Colchicine on Serum Triglycerides in Non-Triton-Treated Rats

Colchicine per 100 g body weight	Serum triglycerides in			
	Sucrose fed*	Change	Purina fed	Change
mg	*mg/dl*	%	*mg/dl*	%
None	218 ± 29		98 ± 11	
0.05	104 ± 12	−52.3	54 ± 8	−44.9
0.50	55 ± 5	−75.0	—	

Colchicine was injected intraperitoneally at time 0 and the experiment was terminated 270 min later.
* Rats supplemented for 5 days with 10% sucrose in their drinking water.

TABLE I

Effects of Colchicine on Secretion of Serum Triglycerides in Female and Male Rats

Colchicine per 100 g body weight	Sex	Serum lipids			
		[^{14}C]Lipid released*	Change	Triglyceride Content	Change
mg		%	%	*mg/dl*‡	%
None	F	48.0 ± 1.9		937 ± 30	
0.05	F	14.4 ± 0.7	−70.0	381 ± 38	−59.3
0.50	F	7.7 ± 2.4	−84.0	336 ± 35	−64.2
None	M	42.3 ± 7.8		540 ± 10	
0.05	M	18.5 ± 4.1	−56.3	253 ± 21	−53.2
0.50	M	10.2 ± 1.1	−75.9	182 ± 18	−66.3

Colchicine was injected intraperitoneally at time 0 and [1-^{14}C]palmitic acid and Triton WR 1339 were injected intravenously at 180 min and the rats were killed at 270 min. Values are means ±SE of five to six rats.

* [^{14}C]Lipid released $\frac{[^{14}\text{C}]\text{lipid in serum}}{[^{14}\text{C}]\text{lipid in liver and serum}} \times 100$; in serum lipids more than 95% of the label was in triglyceride.

‡ dl = 100 ml.

weight the inhibitory action of colchicine was found to be reversible; however, no reversibility up to 7 h could be found at the higher dose level (Table III). Colchicine administration resulted in a similar inhibition of triglyceride secretion in fasted rats, indicating that its effect was primarily on the liver (Table IV). It seemed of interest to compare the action of yet another "mitotic spindle" inhibitor, i.e., vinblastine, on the secretion of serum triglyceride. As seen in Tables V and VI the

TABLE III

Duration of the Effect of Colchicine on the Secretion of Serum Triglycerides

Duration of experiment	Serum lipids, percent of control*			
	[^{14}C]Lipid	Triglyceride	[^{14}C]Lipid	Triglyceride
min				
Colchicine	0.05 mg/100 g body weight		0.5 mg/100 g body weight	
150	69.0 ± 6.0	61.3 ± 5.5	44.6 ± 2.3	51.7 ± 0.6
210	56.7 ± 6.0	50.8 ± 3.4	—	—
270	30.6 ± 1.5	40.6 ± 4.6	17.6 ± 5.5	35.9 ± 3.7
330	40.9 ± 4.8	53.8 ± 4.0	—	—
390	58.8 ± 8.0	68.6 ± 2.6	—	—
450	69.2 ± 10.0	80.2 ± 6.8	10.3 ± 1.3	17.2 ± 3.3

Colchicine was injected intraperitoneally at time 0 and [1-^{14}C]palmitic acid and Triton WR 1339 were injected intravenously 90 min before the termination of the experiment. Values are means ± SE of five to six female rats.
* In control groups of rats used for each time interval studied, the [^{14}C]lipid released into the serum ranged between 43.8 ± 1.31 and 53.6 ± 1.10% of the liver + serum pool (as in Table I), and serum triglyceride levels ranged between 816 ± 10 and 937 ± 30 mg/dl.

TABLE IV

Effect of Colchicine on Secretion of Serum Triglycerides in Fasted Rats

Colchicine per 100 g body weight	Serum lipids			
	[^{14}C]Lipid released*	Change	Triglyceride Content	Change
mg	%	%	*mg/dl*	%
None	45.9 ± 3.9		713 ± 30	
0.05	17.5 ± 2.1	−61.9	326 ± 16	−54.3

The rats were fasted for 16 h and colchicine was injected intraperitoneally at time 0 and [1-^{14}C]palmitic acid and Triton WR 1339 were injected intravenously at 180 min and the rats were killed at 270 min. Values are means ± SE of five to six female rats.
* As in Table I.

TABLE V

Effect of Vinblastine on Secretion of Serum Triglycerides

Vinblastine per 100 g body weight	Serum lipids			
	[^{14}C]Lipid released*	Change	Triglyceride Content	Change
mg	%	%	*mg/dl*	%
None	47.5 ± 4.1		819 ± 57	
0.1	39.2 ± 1.9	−17.5	657 ± 62	−19.5
1.0	25.4 ± 1.5	−46.6	351 ± 24	−57.2

Vinblastine was injected at time 0 and [1-^{14}C]palmitic acid and Triton WR 1339 were injected at 90 min and the experiment was terminated at 180 min. Values are means ± SE of five to six female rats.
* As in Table I.

secretion of triglycerides into the serum was inhibited to a much lesser extent, and the inhibition required a higher dose of vinblastine than of colchicine, which were compared on an equimolar basis. The duration of action of vinblastine was also shorter than that of colchicine and the inhibition was almost completely reversed by 270 min.

To study the secretion of the protein moiety of serum lipoproteins, colchicine-treated and control rats were injected with labeled leucine and killed 60 min thereafter. Results of a representative experiment are shown in Table VII, and it can be seen that while the amount of label incorporated into the sum of liver and serum proteins was similar in both groups of rats, the percent of labeled protein in the circulation was markedly reduced in the colchicine-treated rats. The protein bound serum radioactivity was further characterized by separation into distinct classes of lipoproteins. Results of two representative experiments are presented in Table VIII. Since no Triton was used in these experiments, the concentration of the protein in the various serum fractions examined reflects their rate of turnover. Injection of colchicine resulted in a marked fall in serum levels of VLDL protein but in a much lesser fall in serum HDL protein, while the concentration of protein in the $d > 1.21$ fraction did not change. The inhibition of release of newly synthesized protein was evident in all the serum fractions examined, as seen in the fall in specific activity of the isolated proteins. The fall in specific activity in each class, relative to untreated control (rats), was highest in the $d > 1.21$ fraction, the labeled protein of which consists mainly of serum albumin (21).

In the next series of experiments the effect of colchicine on the rate of bile production and secretion of phospholipid and cholesterol was examined. Since the rats had undergone laparotomy, the drug was administered intravenously. In

TABLE VI

Duration of the Effect of Vinblastine on the Secretion of Serum Triglycerides

Duration of experiment	Serum [^{14}C]lipids (percent of control)
min	
150	74.5 ± 5.9
180	81.5 ± 6.5
210	81.6 ± 6.6
270	92.4 ± 7.8

Vinblastine (0.1 mg/100 g body weight) was injected at time 0 and [1-^{14}C]palmitic acid and Triton WR 1339 were injected 90 min before the termination of the experiment. Values are means ± SE of five to six female rats.

TABLE VII

Incorporation of [^{3}H]Leucine into Liver and Serum Proteins in Colchicine-Treated Rats

Treatment	[^{3}H]Protein in liver (L) + serum (S)	[^{3}H]Protein released*
	dpm × 10^{-3}	%
None	1,287 ± 51	29.6 ± 1.1
Colchicine	1,394 ± 74	12.5 ± 1.4

Female rats were injected with colchicine (0.5 mg/100 g body weight) at time 0 and with [^{3}H]leucine at 180 min and the experiment was terminated at 240 min. Values are means ± SE of five to six rats.

* $\frac{S}{L+S} \times 100.$

TABLE VIII

Effect of Colchicine on Protein Content and Specific Activity of Serum Fractions Separated by Flotation

		Protein in serum fractions			Specific activity of protein in		
Treatment	Exp.	VLDL ($d < 1.006$)	HDL (d 1.085-1.21)	$d > 1.21$	VLDL ($d < 1.006$)	HDL (d 1.085-1.21)	$d > 1.21$
		mg/dl	*mg/dl*	*g/dl*	*dpm/mg* × 10^{-3}		
None	I	6.0	42	4.3	49	9.9	1.5
Colchicine	I	1.2	32	4.6	26	4.4	0.5
None	II	7.4	38	3.9	58	20.0	2.8
Colchicine	II	1.5	24	4.0	27	9.5	0.9

Colchicine 0.5 mg/100 g body weight was injected intraperitoneally at time 0 and [^{3}H]leucine (intravenously) at 180 min; serum was drawn at 240 min; sera from 20 female rats were pooled in each experiment.

TABLE IX

Bile Flow, Phospholipid, and Cholesterol Secretion in Normal and Colchicine-Treated Rats

Treatment	Time	Bile flow	Lipid phosphorus in 2 h bile		Cholesterol in 2 h bile	
	h	*ml*	*μg*	%	*μg*	%
None	0–2	1.65 ± 0.07	136 ± 11.2	100	404 ± 39.5	100
Colchicine	2–4	1.60 ± 0.06	122 ± 14.2	89.7 ± 10.4	402 ± 57.2	99.5 ± 14.2
Colchicine	4–6	1.41 ± 0.09	87 ± 15.8	64.0 ± 11.6	297 ± 33.0	73.5 ± 8.2
None	0–2	1.70 ± 0.1	159 ± 14.5	100	413 ± 57.2	100
None	2–4	1.75 ± 0.1	115 ± 6.1	72.3 ± 3.8	347 ± 21.4	84.0 ± 5.2
None	4–6	1.67 ± 0.1	81 ± 6.2	50.9 ± 3.9	258 ± 21.0	62.5 ± 5.1

Bile ducts were cannulated at time 0. Colchicine (0.2 mg/100 g body weight) was injected intravenously at 2 h and the experiment was terminated 4 hr after injection of colchicine. Values are means ± SE of seven to eight female rats per group.

all animals the first 2-h collection period was considered as 100% base line, and the data obtained in the subsequent two periods were related to this base line. Even though a relatively high dose (0.2 mg/100 g body weight) was used, colchicine did not change appreciably the rate of bile flow during the two periods of collection extending over 4 h (Table IX). Secretion of phospholipid and cholesterol was reduced in the last 2-h period (4–6 h from the start of bile collection), but this reduction was even slightly more prominent in the uninjected controls. Thus it seems that colchicine administration did not affect the secretion of lipids into the bile.

Ultrastructural Findings

After injection of 0.5 (Fig. 1) or 0.05 mg/100 g body weight (Figs. 2 and 3), the general ultrastructure of the hepatocytes remained well preserved, and in some cells a number of cytoplasmic lipid droplets was also seen (Fig. 1). The prominent finding was the accumulation of secretory vesicles containing nascent VLDL particles. The Golgi apparatus was also filled with the particles, and many of the vesicles were seen in the Golgi region. Even though some of the vesicles were seen also in the vicinity of the sinusoidal cell surface (Figs. 3 and 4) there were few or no VLDL particles in the space of Disse, while they were quite numerous in control livers (Fig. 5). The particle-filled secretory vesicles reached quite large dimensions and in some regions they appeared in close proximity to one another (Fig. 6). Small vesicles, also of the coated variety, were seen in the proximity of the secretory vesicles, some of which contained particles that have lost their distinct contours (Fig. 6). Secondary lysosomes with electron-opaque material were often seen in the vicinity of such collections of secretory vesicles as shown in Fig. 6. These findings might suggest increased intracellular breakdown of secretory lipoproteins in the face of inhibition of normal release into the circulation. Whether the inhibition of lipoprotein release by colchicine was mediated through its action on the microtubular system was difficult to determine. In the normal hepatocyte, microtubules are not very numerous but can usually be demonstrated with relative ease (Fig. 7). They were much more difficult to find in the colchicine-treated livers in which bundles of microfibrils were frequently observed in the vicinity of clusters of secretory vesicles (Fig. 8). After incubation of liver slices in the presence of vincristine or vinblastine, typical microcrystalline structures were seen only in Kupffer cells (Fig. 9), while in the hepatocytes the only pertinent finding were bundles composed of fibrillar material (Fig. 10).

DISCUSSION

Serum VLDL are among the main secretory products of the liver cell, and their identification in the hepatocyte has been well documented (1–3). Their intracellular transport has been traced from the site of their synthesis in the endoplasmic reticulum, through the Golgi apparatus and secretory vesicles to the sinusoidal cell surface, and to the space of Disse (1). The transport to the Golgi apparatus appears to be energy dependent, and is interrupted after injection of ethionine, which causes a marked fall in cellular ATP (22). The

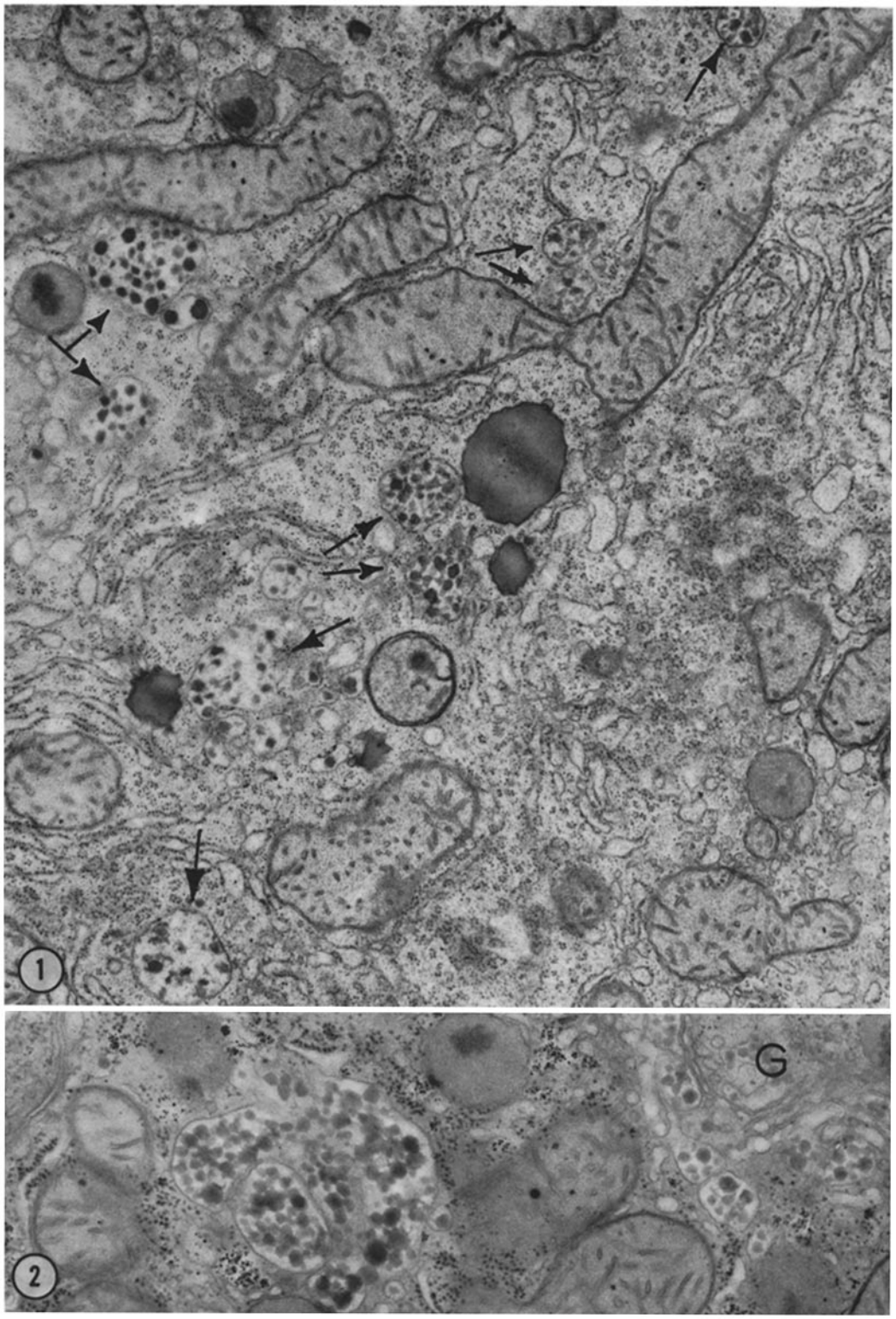
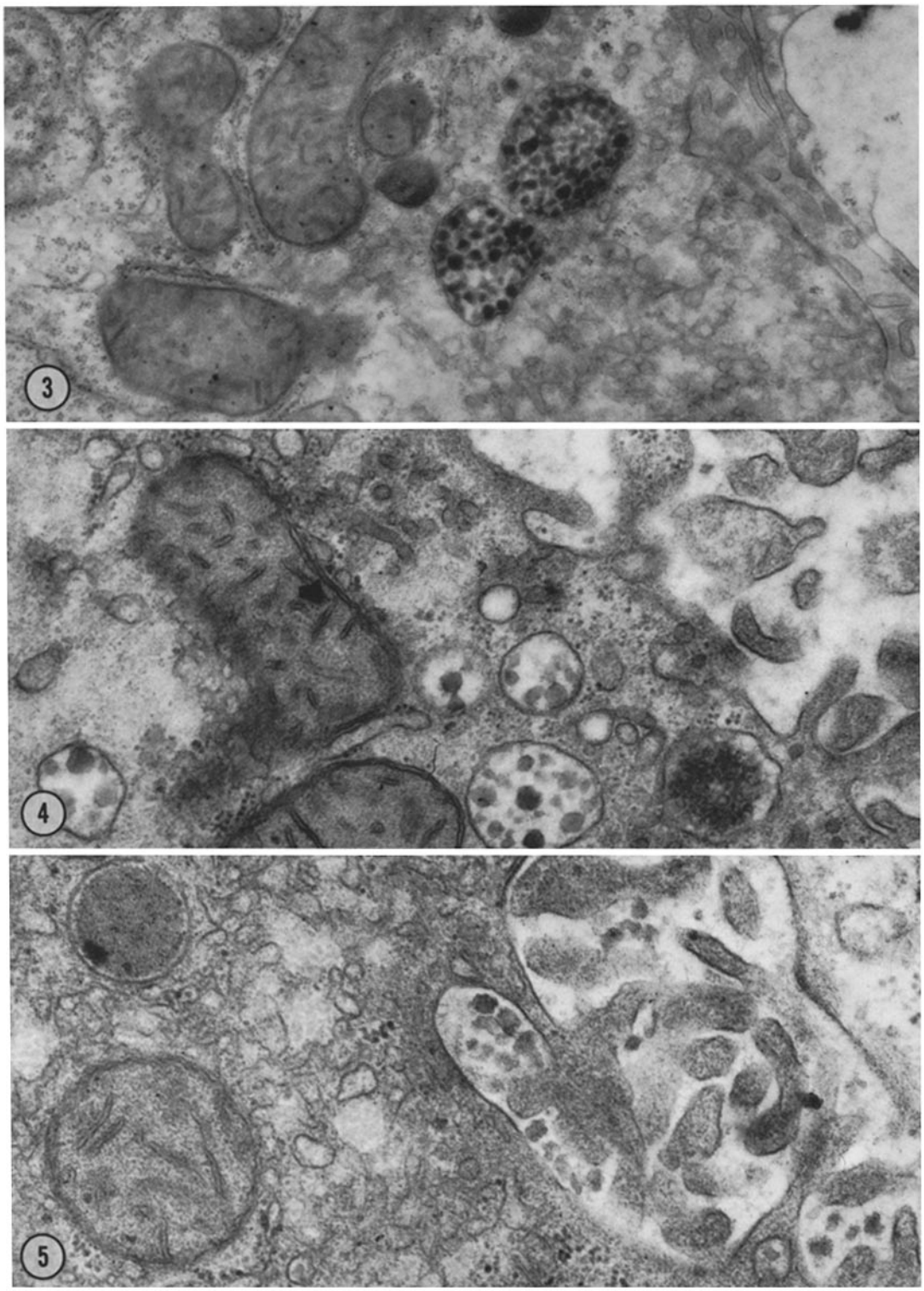


FIGURE 1 Section of rat liver 4 h after injection of colchicine (0.5 mg/100 g body weight). There are numerous secretory vesicles containing nascent VLDL particles (arrows). × 20,000.

FIGURE 2 Section of rat liver 3 h after injection of colchicine (0.05 mg/100 g body weight) showing Golgi apparatus (*G*) and secretory vesicles filled with nascent VLDL particles. × 31,000.



FIGURES 3 and 4 Sections of rat liver 4 h after injection of colchicine 0.05 (Fig. 3) or 0.5 mg/100 g body weight (Fig. 4) showing secretory vesicles containing nascent VLDL particles in the proximity of the sinusoidal cell surface and lack of particles in the space of Disse. Fig. 3, × 20,200; Fig. 4, × 37,500.

FIGURE 5 Section of control liver showing VLDL particles in the space of Disse. × 46,000.

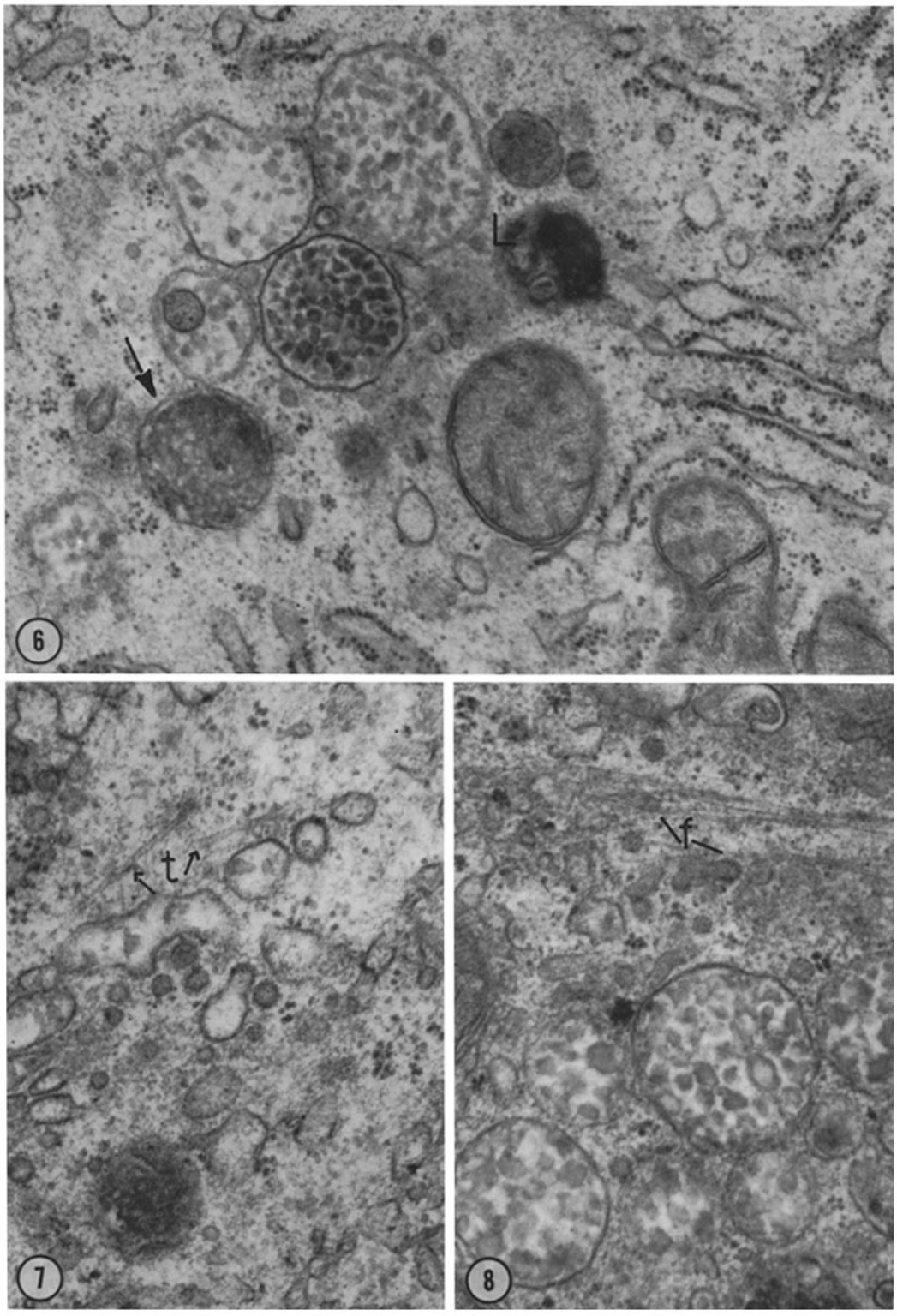
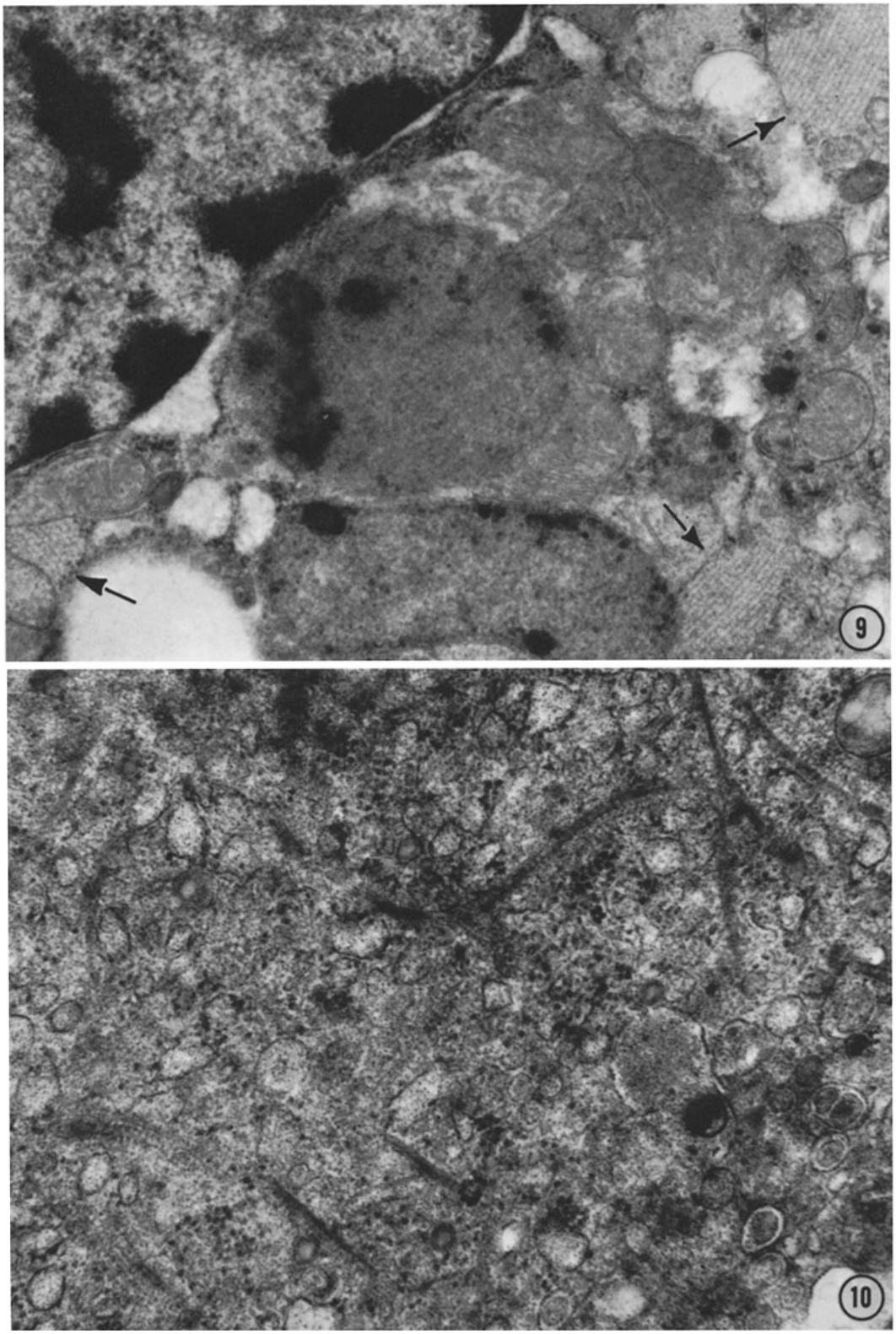


FIGURE 6 Section of rat liver 4 h after injection of colchicine (0.5 mg/100 g body weight). A group of secretory vesicles in close proximity to each other. Nearby are seen small vesicles and a secondary lysosome (*L*). In one of the vesicles (arrow), the particles have lost their distinct outlines. × 39,000.

FIGURE 7 Section of control rat liver showing two microtubules (*t*). × 51,500.

FIGURE 8 Section of rat liver 4 h after injection of colchicine (0.5 mg/100 g body weight). A group of microfilaments (*f*) is seen in the vicinity of secretory vesicles. × 62,000.



FIGURES 9 and 10 Sections of rat liver slices incubated with 10^{-5} M vinblastine (Fig. 9) and with 10^{-5} M vincristine (Fig. 10). In Fig. 9 there are microcrystalline formations in the Kupffer cell (arrows). × 31,000. In Fig. 10, bundles of fibrillar material are seen in the cytoplasm of a hepatocyte. × 62,000.

results obtained with colchicine and vinblastine in the intact rat and with colchicine and vincristine in perfused mouse liver (5, 6) support the possibility that intact microtubules might be required for the operation of the final steps of the secretory cycle.

In the present study this hypothesis, concerning the regulation of secretory processes in the liver, was tested in the intact animal rather than in an isolated organ. Such an approach has its advantages by providing more physiological conditions, but requires certain additional controls, not necessary for the in vitro system. Since, in order to measure the rate of triglyceride secretion, use was made of Triton WR 1339 which was shown to cause a linear increase in serum triglycerides by preventing their removal from the circulation (20), it was important to show that colchicine acts also in non-Triton-treated rats. The second problem posed by the intact animal was that, in addition to the liver, the intestine as well may contribute triglyceride to the circulation. However, since the inhibitory effect of colchicine on triglyceride secretion was similar in fed and fasted rats (Tables I and IV), and since in the latter the intestine has been shown to contribute only about 10% of serum triglyceride (23), it seems plausible that the effect of colchicine was derived primarily by its action on the liver. This is supported also by the finding that, 90 min after injection of Triton, there were 56 mg of triglyceride in 8 ml of serum (4% of body weight) in the controls and only 25 mg in the colchicine-treated rats (Table IV), and thus 30 mg less triglyceride were delivered into the circulation as a result of colchicine treatment. Under fasting conditions the triglyceride secreted by the intestine is derived mainly from hydrolyzed bile phospholipid (24), and under our experimental conditions the amount of phospholipid derived from the bile during 90 min could provide not more than 3 mg of triglyceride (Table IX). Thus it seems that the major part of the missing triglyceride was due mainly to the lack of secretion of hepatic triglyceride. There was an accumulation of the nonsecreted triglyceride in the liver cell inside secretory vesicles. This contributed towards the finding that the degree of inhibition of secretion by colchicine was higher when expressed as percent release of labeled triglyceride, rather than as serum levels of chemically determined triglyceride (Table III).

The main advantage of the intact animal system was that enough serum could be obtained to study the effect of colchicine on the secretion of different serum lipoproteins and to deal with the question whether the final steps of secretion of HDL and of albumin into the serum are regulated in the same manner as those of VLDL secretion. While nascent VLDL have been visualized in the Golgi apparatus (1–3), isolated, and defined by chemical and immunochemical methods (25, 26), the presence of HDL in the Golgi apparatus has been supported mainly by immunochemical means (27). Studies with orotic acid have indicated that HDL secretion by the liver might be independent of VLDL secretion, the latter being inhibited to a much larger extent (28). However, interference with VLDL secretion by orotic acid occurs before the Golgi apparatus, as evidenced by the accumulation of liposomes in the cisternae of the endoplasmic reticulum (29, 30). The localization of the intrahepatic precursor of serum albumin in the Golgi apparatus was first suggested by Bruni and Porter (31) and has obtained support by the studies of Ashley and Peters (32) and Glaumann and Ericsson (33). Our present findings indicate that the final pathways of intracellular transport and secretion of VLDL, HDL, and most probably serum albumin (present in the $d > 1.21$ fraction) are subject to a common regulatory mechanism.[1] The interruption of the final steps of secretion resulted in the accumulation of the secretory vesicles, which were seen throughout the cytoplasm of the liver cell, but it still remains to be shown whether all three products are present in the same vesicle, or are segregated among different vesicles. It seems important to point out that the maximal decrease in specific activity observed in the $d > 1.21$ fraction was not due to a preferential inhibition of its release, but rather to the dilution of newly secreted labeled molecules in a pool of albumin, the $t_{1/2}$ of which is about 3 days (35) as compared to 10 h for HDL (36) and several minutes for VLDL (37). One of the theories proposed for the role of microtubules in the secretory process was that these organelles act as guidelines for the movement of the secretory vesicles towards the plasma membrane (38). The present finding of secretory vesicles in the vicinity of the plasma membrane could suggest that colchicine treatment interfered with

[1] Recently, accumulation of albumin in liver slices after vinblastine and colchicine treatment was described by Redman (34).

the release of the secretory product, rather than with its transport towards the secretory pole. However, one cannot rule out the possibility that colchicine has interfered with the regulation of a random migratory process, which might depend in part on microtubules to provide pathways for the secretory vesicles. If one may extrapolate from the data on *Tetrahymena* to the liver cell, one could envisage that fusion of the secretory vesicle with the plasma membrane can occur at certain specific recognition sites (39). Ukena and Berlin (40) have shown that in polymorphonuclear leukocytes and macrophages the cell surface operates as a functional mosaic in which specific transport sites are separated from those parts of the membrane that are usually internalized by phagocytosis. This topographic separation was no longer demonstrable after treatment with colchicine and vinblastine (40). These agents inhibited also the agglutination of polymorphonuclear leukocytes by concanavalin A (41). Since agglutination seems to be related to a certain distribution of specific sites on the cell surface, it was suggested that these sites assume a more homogeneous distribution in the absence of normal microtubular function (41). These results would indicate that the function of the microtubules in the secretion of lipoproteins and proteins from the liver is related to the maintenance of the specific organization of the plasma membrane which is mandatory for the normal process of fusion between the membranes of the secretory vesicles and the plasma membrane. One should also consider the possibility that the interference with the release of the secretory product could be due to colchicine binding to cellular membranes as has been recently shown by Stadler and Franke (42). It seems of interest that, by analogy to the findings in hamster kidney fibroblasts (43), the loss of microtubules was accompanied by an apparent increase in microfilaments in the hepatocytes of colchicine-treated rats and in liver slices incubated with vincristine.

Our results concerning the change in the rate of secretion of phospholipid and cholesterol into the bile with time of cannulation agree with those of Baxter (24). The present study has also provided some new insight concerning the secretion of bile phospholipid and cholesterol. The studies performed so far did not provide information as to the ultrastructure of the precursor pool of biliary phospholipid, the site of synthesis of which has been localized to the endoplasmic reticulum (17). Even though flattened disks, composed of a bilayer and probably corresponding to cholesterol-lecithin-bile acid micelles, have been demonstrated in the bile (44), such structures were not identified within the hepatocytes, even in biliary obstruction (45). The finding that colchicine did not inhibit secretion of lecithin or cholesterol into the bile adds support to the concept that biliary lipids are not secreted in the form of particles or granules carried to the secretory surface in membrane-bounded vesicles. This is in good agreement also with the lack of inhibition by colchicine and vinblastine of steroid secretion from adrenocortical tumor cells in culture, which apparently is also not released in the form of secretory granules (46).

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