EVIDENCE THAT MICROTUBULES PLAY A PERMISSIVE ROLE IN HEPATOCYTE VERY LOW DENSITY LIPOPROTEIN SECRETION

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ABSTRACT

To determine whether a minimum number of assembled microtubules is required for very low density lipoprotein (VLDL) triglyceride (TG) secretion in hepatocytes, antimicrotubule drugs of different concentrations were given to rats. Hepatic VLDL-TG release was subsequently measured by a liver perfusion system, and hepatocyte ultrastructural changes were analyzed by quantitative ultrastructural methods. The results demonstrate a tight coupling between the reduction in hepatocyte microtubule content and the reduction in hepatic VLDL-TG secretion which is related to the dose of colchicine or vinblastine administered. The various estimates imply that a minimum number of microtubules is necessary for hepatic VLDL secretion to proceed normally and that hepatic VLDL secretion rates reach their nadir (10–30% of control) when microtubules comprise <0.005% of the cytoplasm (or <10% of control values). At this point, hepatocyte Golgi complexes are also greatly altered; Golgi complexes with recognizable dictyosomal membranes are reduced to 15% of control values and the region is filled with large numbers of electron-dense bodies which appear to be lysosomes in the process of digesting VLDL. There is a predilection for the remaining Golgi complexes to be associated with a few segments of microtubules, even when no microtubules can be measured in random samplings of hepatocytes. Clusters of vacuoles containing VLDL are also present throughout the cytoplasm; the limiting membranes of 25% of these vacuoles are studded with ribosomes. These findings demonstrate that the administration of antimicrotubule agents results in decreases in hepatic VLDL-TG secretion which are associated with loss of microtubules and alteration of existing Golgi complexes.

KEY WORDS microtubules · hepatocyte · colchicine · very low density lipoprotein secretion · vinblastine · Golgi complexes

Hepatocytes synthesize and secrete triglycerides primarily in the form of very low density lipoproteins (VLDL). Recent studies from our laboratory (14) demonstrated that both acute and sustained increases in VLDL secretion can occur without measurable changes in the hepatocyte content of either the assembled or disassembled form of microtubule protein. This observation raised certain issues concerning the role of microtubules in VLDL secretion, and led to the present study in

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which three questions were considered. One, can VLDL secretion occur in the absence of assembled microtubules? Two, is there a minimum number of assembled microtubules required for VLDL secretion? Three, when microtubules are absent, what structural changes, if any, occur in hepatocytes? To help answer these questions, antimicrotubule drugs of different concentrations were given to rats. After various periods of time, hepatic VLDL release was measured in a liver perfusion system, and hepatocytes were examined for ultrastructural changes.

MATERIALS AND METHODS

Animal Protocol

Young (250–350 g) male Sprague-Dawley rats were used for all studies. The rats were fed standard laboratory chow (Wayne Lab Blox, Allied Mills, Inc., Chicago, Ill.) and were maintained on a 12-h light and dark (6 a.m./6 p.m.) cycle. On the morning of an experiment, food was removed from the animals, and 1 h later the rats were injected with saline or freshly prepared colchicine (Sigma Chemical Co., St. Louis, Mo.) (0.05–2.5 mg/kg body weight), vinblastine sulfate (gift from R. M. Hosley, Eli Lilly and Co., Indianapolis, Ind.) (0.5–5.0 mg/kg body weight), or lumicolchicine (prepared as described by Mizel and Wilson [7]) (2.5 mg/kg body weight). In general, two animals were studied each day: one rat was injected with either colchicine, vinblastine, or lumicolchicine, while the second rat was injected with saline. 3 h later the rats were anesthetized with sodium thiamylal (40–50 mg/kg), and the livers were perfused *in situ* (by methods previously described in detail [14]) with oleic acid (0.7–0.8 mEq/liter) as an exogenous substrate source for VLDL-triglyceride (TG) synthesis. Timed samples of perfusate were removed over a 2-h period, and TG concentrations were measured (1). VLDL-TG secretion rates were calculated from knowledge of these data and the perfusate volume.

Immediately after the 2-h perfusion, each liver was briefly flushed with a cacodylate/sucrose buffer (7% sucrose in 0.1 M cacodylate; 22°C; pH 7.2; 350 mosmol) and perfused for 5 min with 2% glutaraldehyde in 0.1 M cacodylate buffer (22°C; pH 7.2; 420 mosmol). Liver samples from the left lobe were minced and submerged in the fixative for three additional hours at 22°C. Thereafter, the tissue samples were washed two times with the sucrose/cacodylate buffer, and left in buffer overnight at 4°C. Further processing of the tissue was continued the next day.

Ultrastructural Morphometric Methods

GENERAL PROCEDURE: Fixed and washed tissue samples were subsequently postfixed for 1 h in 1% osmium tetroxide (veronal buffer; pH 7.2; 22°C) and stained en bloc for 45 min (2% uranyl acetate in veronal buffer, pH 5.5; 22°C) before dehydration in graded alcohols and embedment in Epon-Araldite plastic. Thick sections which revealed portal areas were subsequently thin sectioned (400–500 Å), stained with uranyl acetate and lead citrate, and viewed with a JEM-100 B (Jeol Co., Burlingame, Calif.) electron microscope.

ASSESSMENT OF MICROTUBULE CONTENT IN HEPATOCYTES: These procedures have been described in detail in previous publications (11, 12, 14). In brief, six nucleated hepatocytes chosen at random from one block of each rat liver were photographed in the 12 o'clock and 6 o'clock positions at × 16,000. Care was taken to include the nuclear membrane in one picture and the plasma membrane in the second picture of each cell. These electron micrographs were photographically enlarged to × 48,000. All microtubule lengths and cross sections were identified on the prints, and microtubule volume density relative to cytoplasmic volume density was estimated by point-counting stereological techniques (19).

CONTENT OF VARIOUS CYTOPLASMIC ORGANELLES IN HEPATOCYTES: Each hepatocyte photographed for the study of microtubules was also photographed at × 3,000 (to include the profile of the entire cell) and at × 8,000. The lowest magnification photographs were used to assess changes in the distribution of various organelles; higher magnification photographs were used to estimate the content of Golgi complexes and lysosomes, and the distribution of hepatocyte VLDL. For these purposes, Golgi complexes were considered to be "intact" when at least one dictyosome cisterna was found associated with coated and smooth vesicles and/or VLDL-containing vacuoles. Lysosomes were identified as electron-dense bodies containing fragments of cellular structures in various stages of digestion. VLDL were separately quantitated in: (*a*) Golgi vacuoles associated with Golgi complexes; (*b*) smooth (SER) or rough-surfaced endoplasmic reticulum (RER); and (*c*) lysosomes.

ASSESSMENT OF MICROTUBULE ASSOCIATIONS: Postfixation microtubule association with other structures of the cell was evaluated by a modified version of the "random circle" morphometric method developed in this laboratory (13). This method is based on the premise that microtubule cross sections are clearly identifiable at the magnification used (× 48,000), and that one can quantify the proximity of these cross sections to various organelles on micrographs and compare the value with one obtained by quantifying the centers of random circles (drawn by a computer) on the same pictures.[1] In the present study we assessed the proximity of microtubule cross sections to the following three secretory organelles: Golgi complexes, VLDL-containing vacuoles (not associated with Golgi complexes), and plasma membranes.

The total number of microtubules associated with the Golgi complex was analyzed by an independent method as follows: Golgi areas were sought in otherwise randomly selected cells and photographed at × 16,000. Intact Golgi regions (Golgi cisternae and associated vesicles and/or VLDL-containing vacuoles) were identified on threefold magnified photographic prints of the original micrographs, and their general outlines were encircled with a pencil. A second line, separated from the first by 1 cm,[2]

[1] Cross sections of microtubule were used exclusively in this study to eliminate arbitrary decisions as to which part of a microtubule longitudinal segment one should use in the analysis (e.g., the center or either end point). Although it is possible that some microtubule cross sections are not correctly identified in photographs of hepatocytes, errors in identification occur without bias, i.e., microtubule cross sections near Golgi membranes have as much opportunity to be correctly or incorrectly identified as microtubule cross sections near plasma membranes. As a result, we believe that no systematic error is introduced into the analysis.

[2] This distance was arbitrarily chosen after inspection of numerous prints of Golgi regions; at × 48,000, many microtubules, which seem to relate to Golgi areas, are not actually within the complexes but close to them.

was then drawn following the contours of the first line. All microtubule lengths or cross sections found within the area enclosed by the second line were considered to be associated with the Golgi complex; all other microtubule segments within the micrographs were considered not associated with the Golgi complex. Each microtubule segment, regardless of length, received one count unless it traversed both regions, in which case it was counted in each region. Subsequently, the total area enclosed by the Golgi regions (plus the additional 1 cm) and non-Golgi cytoplasmic regions was estimated so that the number of microtubules observed in each region could be assessed per unit area.

RESULTS

Effect of Antimicrotubule Agents on VLDL-TG Secretion Rates

COLCHICINE: Fig. 1 indicates that VLDL-TG secretion from perfused livers of control (saline) rats is 2.3 mg/h per gram liver. Hepatic VLDL-TG secretion rates do not decrease 3–5 h after the administration of 0.15 mg colchicine/kg body weight, but colchicine in a dose of 0.3 mg/kg leads to a fall in mean VLDL-TG secretion rate to 70% of the control values ($P < 0.01$). At a colchicine dose of 0.5 mg/kg, VLDL-TG secretion rates fall to ~30% of control values ($P < 0.01$). Further increases in the amount of administered colchicine lead to only relatively small decrements in the absolute hepatic VLDL-TG secretion rate. The use of lumicolchicine at a dose equivalent to the highest colchicine dose (2.5 mg/kg) does not alter VLDL-TG secretion rates.

VINBLASTINE SULFATE: Like colchicine, vinblastine administration also leads to a dose-related depression of VLDL-TG secretion; in this instance, 0.5 and 1 mg vinblastine/kg do not affect TG secretion; 2.5 mg/kg vinblastine reduces TG secretion to 70% of control values, and 5.0 mg vinblastine/kg reduces TG secretion to 30% of control values.

Effect of Antimicrotubule Agents on Hepatocyte Microtubule Content

COLCHICINE: Fig. 2 shows hepatocyte microtubule volume density at the end of the perfusion experiments (or 5 h after the injection of either saline, colchicine, or vinblastine). When no colchicine is given (saline), microtubule volume density is ~0.03% of the cytoplasm. This value does not change significantly until colchicine is given to rats at a dose of 0.3 mg/kg; at this point there is a striking fall in microtubule volume density to ~15% of control values. Hepatocyte microtubule content decreases almost to zero at a dose of 0.5 mg colchicine/kg. A fivefold increase in administered colchicine leads to a further small reduction in microtubule volume density. Lumicolchicine, on the other hand, does not alter normal hepatocyte microtubule volume density, even when administered at a dose of 2.5 mg/kg body weight. Since tissues were obtained at the end of the perfusion period (5 h after colchicine), it was of interest to determine the microtubule content of cells 3 h after colchicine treatment (a stage just before the start of perfusion). These studies were

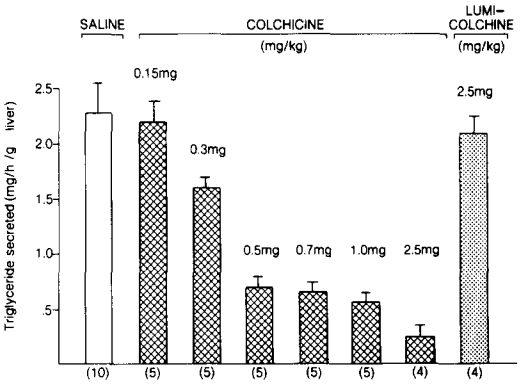

FIGURE 1 Hepatic VLDL-TG secretion rates observed after increasing doses of colchicine. Data are expressed as mean (± SEM) secretion rates 3–5 h after the administration of saline (clear bar), colchicine (hatched bars), or lumicolchicine (stippled bar). Figures in parentheses represent the number of livers perfused at each drug dose.

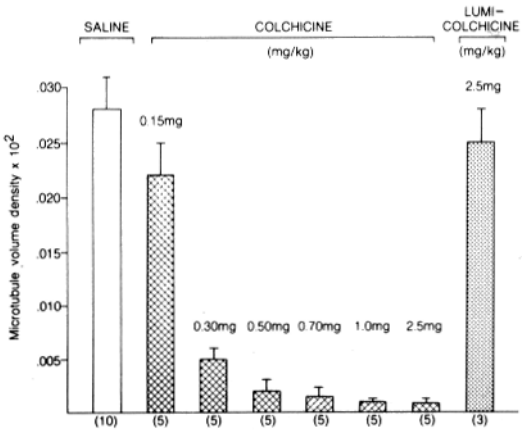

FIGURE 2 Hepatocyte microtubule content 5 h after the administration of various doses of colchicine. Data are expressed as mean (± SEM) volume density of microtubules. The key is the same as in Fig. 1. Figures in parentheses represent the number of rats from which tissue was analyzed at each given dose of colchicine.

performed with an administered colchicine dose of 0.7 mg/kg, and the results of six studies indicate that there is no difference in the microtubule content at 3 and at 5 h after the administration of this dose of colchicine.

VINBLASTINE SULFATE: The administration of vinblastine results in a similar decline in hepatocyte microtubule content. When given to three rats at 1 mg/kg, vinblastine has no effect on hepatocyte microtubule content (mean ± SEM volume density of microtubules is 0.030 ± 0.001; at 2.5 mg/kg (n = 3), the content of microtubules drops to 50% of control values, and at 5.0 mg/kg (n = 3), no microtubules can be found in hepatocytes.

Relationship between Changes in VLDL-TG Secretion and Microtubule Volume Density

Fig. 3 directly compares the effect of colchicine on VLDL-TG secretion (Fig. 1) and hepatocyte microtubule content (Fig. 2), and several points can be made from these data. First, there is remarkable similarity in the shape of the two dose-response curves: the range of colchicine in which the most dramatic changes occur in both variables is very small (between 0.15 and 0.5 mg colchicine/kg). Second, despite the general parallelism between the two curves, dissociation between VLDL secretion and microtubule content can be demonstrated after the administration of 0.3 mg colchicine/kg: 5 h after this dose of colchicine, a much greater reduction in microtubule volume density

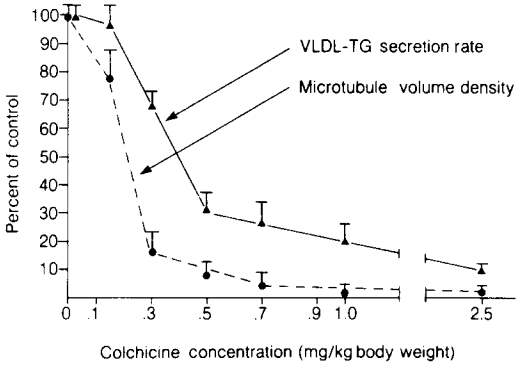

FIGURE 3 Comparison of hepatic VLDL-TG secretory response and hepatocyte microtubule response to increasing doses of administered colchicine. Data are derived from Figs. 1 and 2, and are plotted as percent of the control (saline) value. Triangles represent mean (± SEM) secretory rates; circles represent values for mean (± SEM) microtubule content.

(85%) is seen than in VLDL-TG secretion (30%). Finally, Fig. 3 shows that hepatic VLDL-TG secretion can continue at a low level (10–30% of control values) at a point (i.e., after a colchicine dose of 0.5–2.5 mg/kg) when microtubules are largely absent in cells analyzed by random sampling techniques.

Although the data are not so complete, a similar relationship exists between the dose of administered vinblastine and the VLDL-TG secretion and microtubule content, i.e., VLDL-TG secretion is shown to be significantly ($P < 0.01$) reduced at concentrations of vinblastine that depress microtubule content by 50%. However, as with colchicine, VLDL-TG secretion can continue at 30% of control values at a time when microtubule volume density has been reduced almost to zero.

Effect of Antimicrotubule Agents on the Content of Various Cytoplasmic Organelles

When VLDL-TG secretion and microtubule content are reduced after the administration of colchicine (0.3–2.5 mg/kg) or vinblastine (2.5–5.0 mg/kg), distinct morphological changes appear in hepatocytes. First, vacuoles filled with VLDL appear isolated or in clusters within the cytoplasm (Fig. 4). Although the membranes of many of these vacuoles are associated with ribosomes (Figs. 4 and 5), and appear to be engorged portions of the RER, the majority of the vacuole membranes are smooth surfaced (Figs. 4 and 6).

In addition, variable numbers of lysosomes are seen throughout the cytoplasm (Fig. 4). These electron-dense bodies frequently contain remnants of VLDL which, except for their unusual density, resemble the VLDL-filled vacuoles often associated with Golgi complexes (Fig. 4). Golgi complexes in general appear altered after colchicine treatment. The most apparent change is the large number of lysosomes associated with Golgi components (Figs. 7–9). On closer inspection, it is clear that stacked cisternal membranes are reduced in amount in Golgi regions. Indeed, in cells of animals treated with relatively high doses of colchicine (>0.7 mg/kg), it is rare to come upon an "intact" Golgi complex (that is, a region in which stacked cisternal membranes are associated with VLDL-filled vacuoles and small coated and smooth vesicles).

In an effort to quantitate the observed changes in Golgi complexes, lysosomes (dense bodies), and accumulation of VLDL, various morphometric

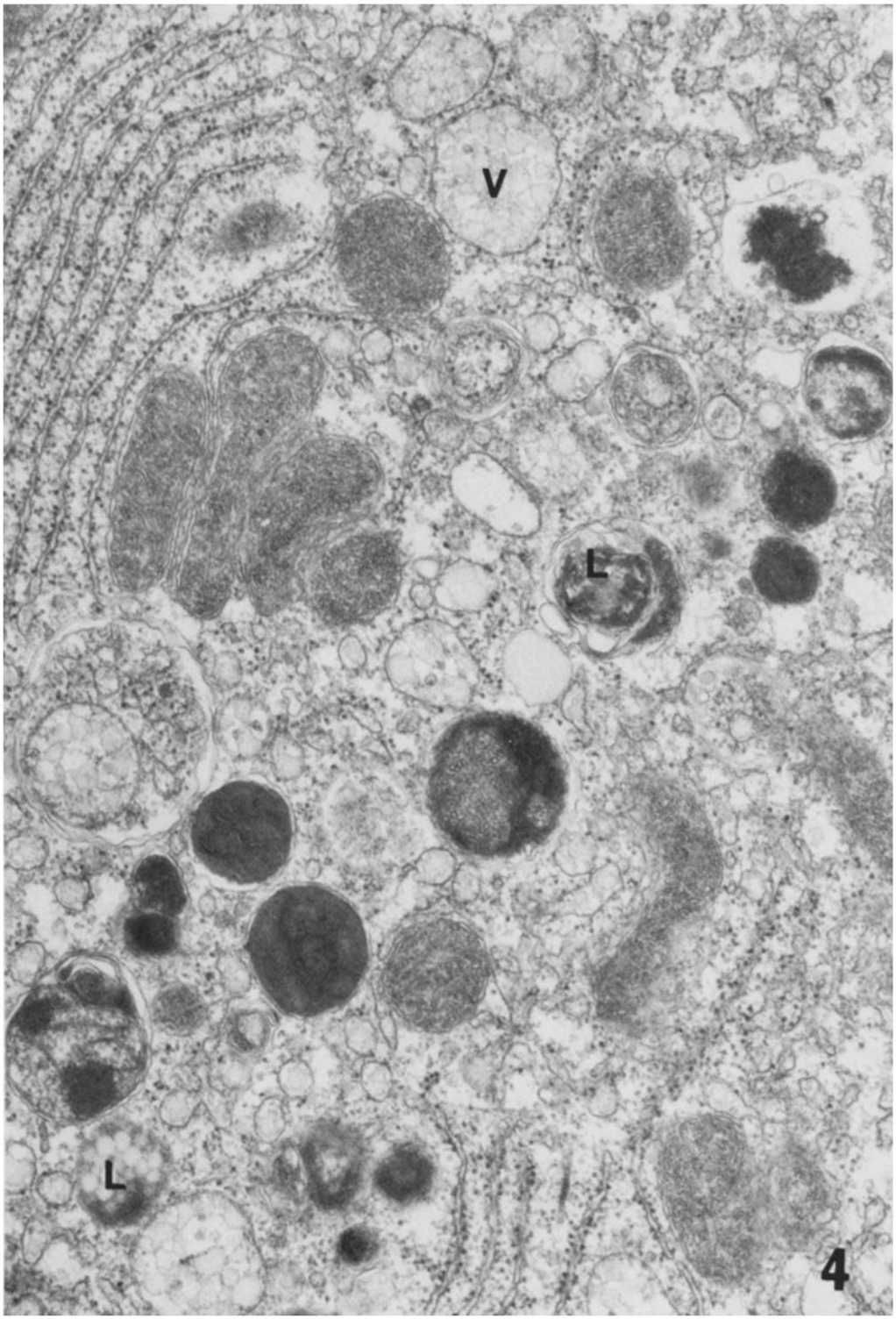

FIGURE 4 Low magnification view of representative portion of hepatocyte cytoplasm from rat injected with colchicine (0.7 mg/kg) 4 h earlier. Cytoplasm shows normal arrangement of RER, SER, and mitochondria, but striking increase in number of lysosomes (*L*) and vacuoles filled with VLDL (*V*). × 35,000.

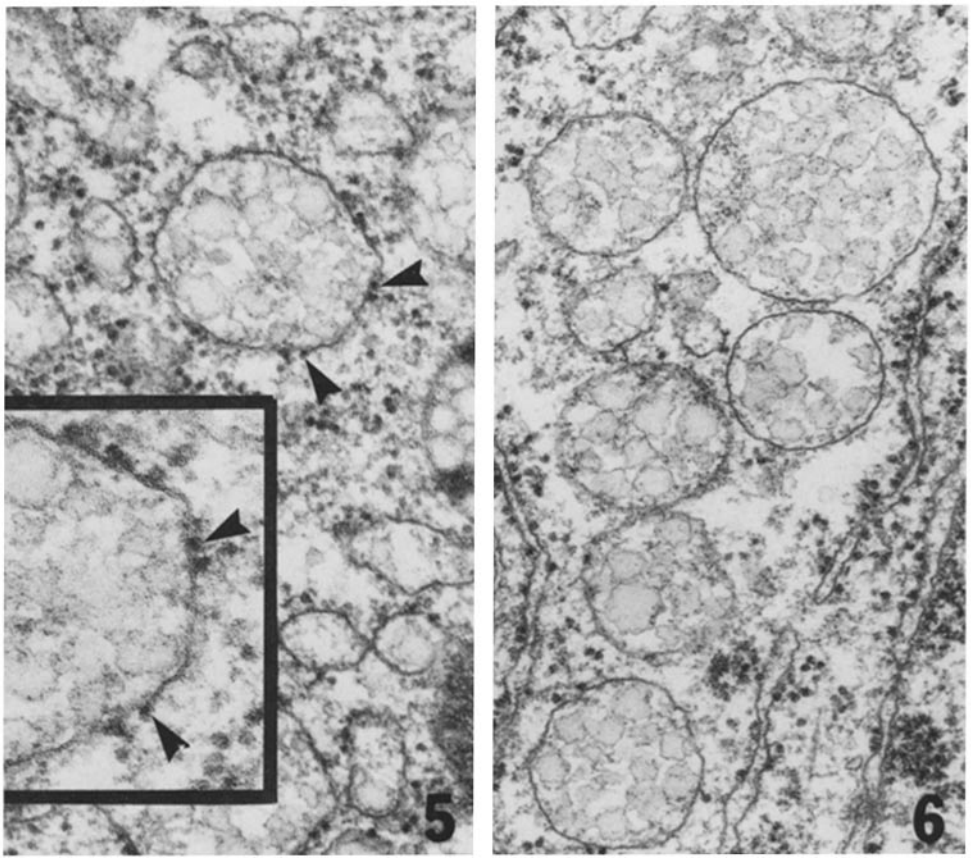

FIGURE 5 Single VLDL-filled vacuole surrounded by membrane-containing ribosomes. Vacuoles of this type are found in rats treated with colchicine (0.3–1.0 mg/kg). × 60,000. *Inset* shows higher magnification view of the same vacuole membrane. × 100,000.

FIGURE 6 Representative cluster of smooth-surfaced vacuoles containing VLDL. This group was from same cell as rough-surfaced vacuole in Fig. 5. × 60,000.

measurements were made on hepatocytes of animals given colchicine. Golgi complexes were evaluated in cells of rats given several different doses of colchicine previously shown to reduce hepatic VLDL-TG secretion and hepatocyte microtubule content (Figs. 1–3). Lysosomes and VLDL-containing organelles were evaluated in hepatocytes of animals given 0.3 mg/kg colchicine, which results in moderate reduction of VLDL secretion, and 0.7 mg/kg colchicine, which results in near maximal reduction of VLDL secretion (Figs. 1 and 3). These results appear in Table I and make the following points: First, intact Golgi complexes comprise slightly more than 2% of normal hepatocyte cytoplasm. When colchicine is given in a dose of 0.3–0.5 mg/kg, the mean volume of Golgi complexes is not statistically different from that of control animals. However, when colchicine is given in a higher dose (0.7–1.0 mg/kg), hepatocytes lose 85% of their normal content of intact Golgi complexes. Although not shown in Table I, the colchicine-induced reduction in Golgi complexes is not selective for special regions of the cells, i.e., after 0.7 mg/kg colchicine, 35% of the remaining intact Golgi complexes are still associated with bile canaliculi as they are in untreated cells. Table I indicates also that hepatocyte VLDL compartmentalization is altered after animals are treated with both 0.3 and 0.7 mg/kg colchicine. In hepatocytes of saline-treated rats, the vast majority of VLDL are associated with Golgi vacuoles within the Golgi compartment. After 0.3 mg col-

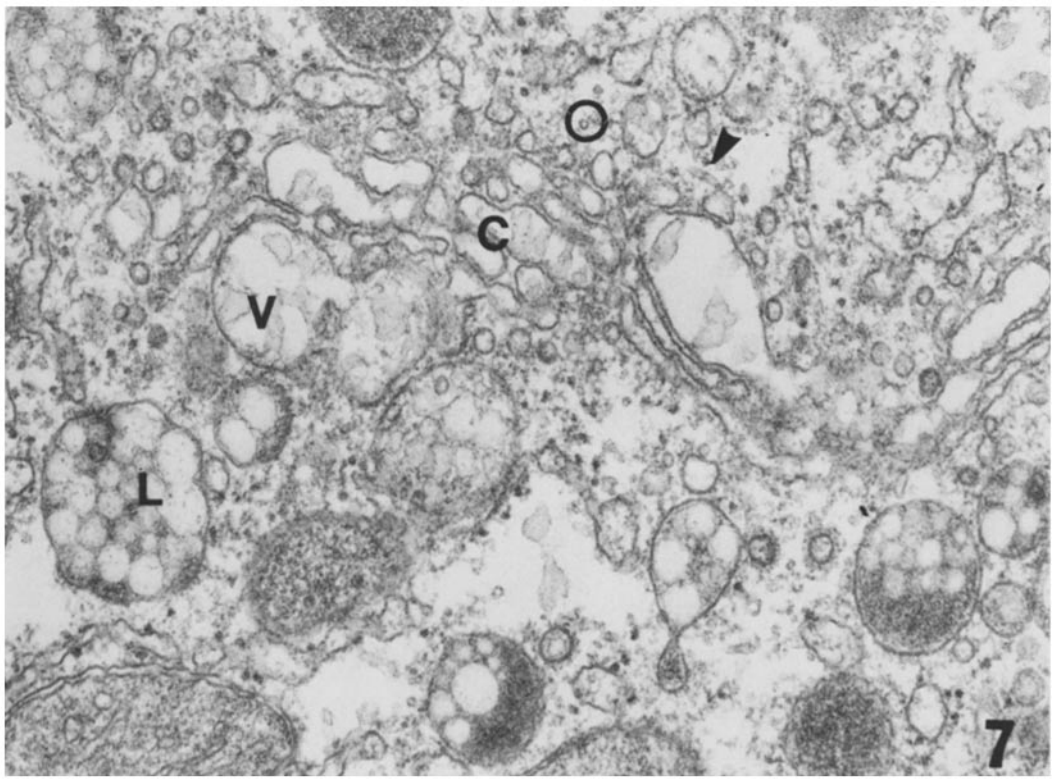

FIGURE 7 An intact Golgi region of hepatocyte of colchicine-treated (0.3 mg/kg) rat. The region shows well-developed dictyosomal cisternae (*C*) and numerus small vesicles of both the smooth and the coated variety. Several vacuoles (*V*) contain VLDL, and some appear to be lysosomes (*L*) containing remnants of VLDL. A microtubule cross section is encircled; microtubule length is indicated by arrowhead. × 60,000.

chicine, the content of VLDL associated with vacuoles in the Golgi compartment is not significantly changed, but increasing amounts of VLDL are associated with lysosomes (Fig. 7) and endoplasmic reticulum. After 0.7 mg/kg colchicine, few intact Golgi remain. As a result, fewer VLDL overall are associated with Golgi complexes (Fig. 8). However, the content of VLDL within lysosomes and within endoplasmic reticulum is increased sevenfold.

Although not shown in Table I, it was found that in cells of saline-treated rats, 95% of the VLDL (not associated with Golgi complexes) are found in SER and only 5% in RER; in cells of colchicine-treated rats (0.7 mg/kg), this ratio changes and only 74% of the non-Golgi VLDL are found in SER, whereas 26% are in RER.[3] Also, VLDL tends to accumulate in extracellular sites (Fig. 9) in colchicine-treated livers, e.g., in livers of rats given 0.5–1.0 mg/kg colchicine, the amount of VLDL found between hepatocytes is 5- to 10-fold that found in livers of saline-treated animals.

These morphological effects of colchicine on hepatocytes are seen also after treatment with vinblastine sulfate. After 5.0 vinblastine/kg, Golgi complexes are reduced by 85% (±10%). Although not specifically measured, the content and distribution of lysosomes and of VLDL in various cellular compartments after administration of 5.0 mg of vinblastine/kg, appear identical to those seen after treatment with 0.7 mg/kg colchicine.

[3] Although images in Figs. 4 and 5 suggest that the membranes of VLDL-filled vacuoles contain ribosomes, it is not always possible (at magnifications convenient for morphometric measurements) to distinguish between a vacuole of this sort and a smooth-surfaced vacuole with a closely applied segment of RER. As a result, the estimate for VLDL in RER membranes may be somewhat inflated.

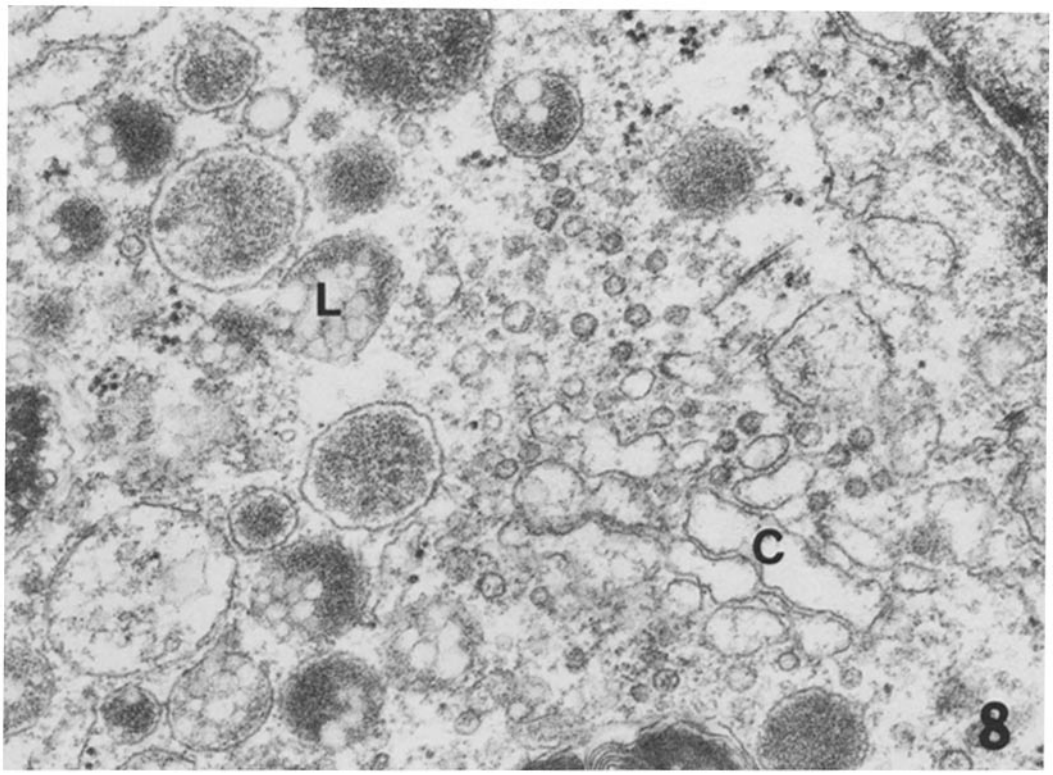

FIGURE 8 Golgi region from colchicine-treated (0.7 mg/kg) rat. Golgi cisternae (*C*) are still apparent in this micrograph (though greatly reduced overall; see Table I). The region contains a great increase in lysosomes (*L*) (many of which include VLDL remnants) and a variety of small smooth and coated vesicles. × 60,000.

Association of Microtubules with Various Organelles of the Cell

When analyzed by the "random circle" method, microtubules in untreated hepatocytes are found to have a special, statistically significant relationship to Golgi complexes ($P < 0.01$), but not to certain other structures (such as lysosomes, VLDL-filled vacuoles, and plasma membrane) thought to be involved in hepatocyte VLDL disposal. When this distribution of microtubules is estimated per unit volume of cytosplasm, it appears that 78% of all microtubules (found in micrographs of Golgi regions of cells of control animals) are closely associated with Golgi complexes. In contrast, there are far fewer Golgi complexes in cells of rats treated with 0.7 mg colchicine/kg (see Table I), and very few microtubules overall (see Fig. 2). Of the remaining microtubules, however, >95% are associated with the remaining Golgi complexes (see Figs. 7 and 9), suggesting a unique, spatial relationship between these structures even after colchicine treatment.

DISCUSSION

These studies demonstrate that the disruption of hepatocyte microtubules that follows the administration of either colchicine or vinblastine is associated with a reduction in hepatic VLDL-TG secretion. The most dramatic effects occur over a very small range of administered drug, i.e., between 0.15 mg colchicine/kg (where no changes are seen in either VLDL-TG secretion or microtubule content) and 0.5 mg colchicine/kg (where near-maximal changes are seen in both TG secretion and microtubule content). Furthermore, there appears to be a striking degree of parallelism between the effect of antimicrotubule agents on hepatocyte microtubule content and the effect on hepatic VLDL-TG secretion (see Fig. 3). The apparent existence of such an exceedingly tight coupling between hepatocyte microtubule content and

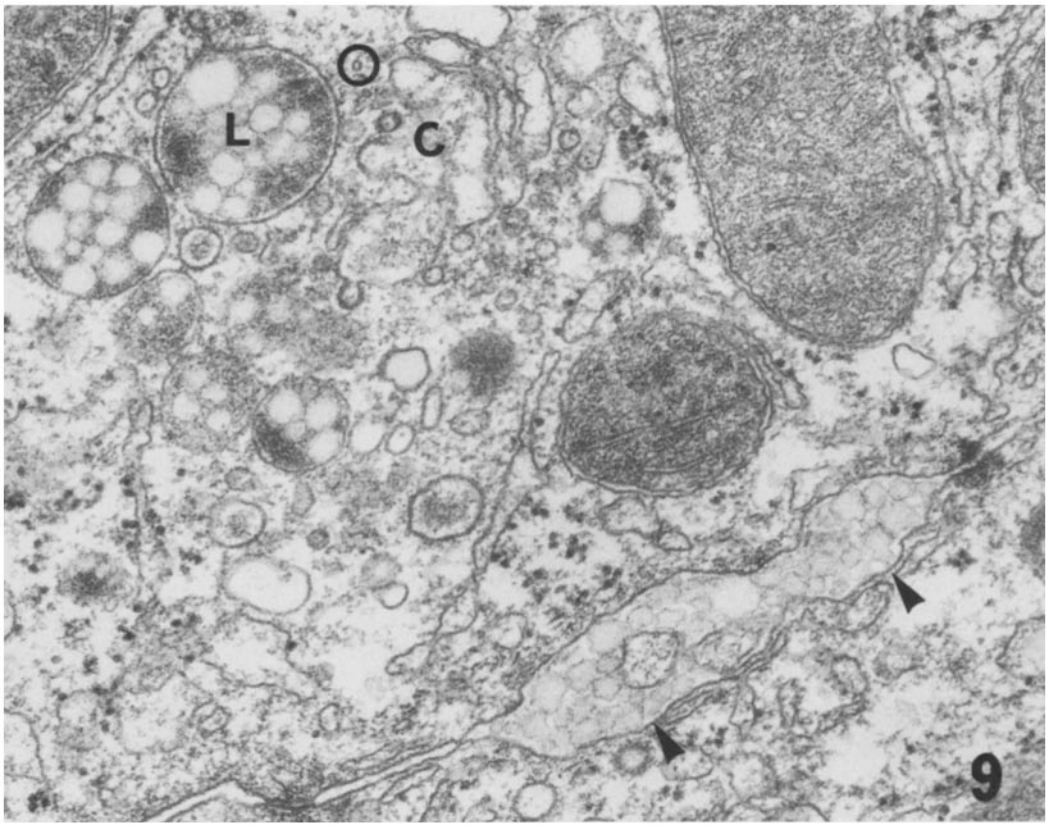

FIGURE 9 Golgi region from colchicine-treated (0.5 mg/kg) rat. Dictyosomal cisternae (*C*) are still present in this micrograph. The region contains a variety of small vesicles and a few lysosomes (*L*) containing remnants of VLDL. A microtubule cross section is encircled. This photograph also shows the abnormal accumulation of VLDL (arrowheads) between hepatocytes (typically in an enclosed space adjacent to a bile canaliculus but bounded by desmosomes). × 60,000.

hepatic VLDL-secretion could imply a functional relationship between the two, and the fact that this is observed after the use of two totally different antimicrotubule agents (colchicine and vinblastine sulfate) adds support for this view. The precise nature of the relationship between hepatocyte microtubule content and hepatic VLDL-TG secretion remains to be defined, but the results presented indicate that near-normal (70% of control) hepatic VLDL-TG secretion can be maintained in spite of a fall in hepatocyte microtubule volume to 15% of control values (see Fig. 3). On the other hand, a further fall of only 5% in hepatocyte microtubule content (to a total of a 90% reduction) leads to a dramatic and near-maximal fall in VLDL-TG secretion. These data suggest that microtubules must comprise at least 0.005% of the cytoplasm to sustain hepatic VLDL-TG secretion.

Although we have emphasized the tightly coupled nature of the fall in hepatocyte microtubule content and in hepatic VLDL-TG secretion that occurs when several different doses of colchicine and vinblastine are given, these data also demonstrate a dissociation in the response of these two variables to a critical level of colchicine. Thus, a dose of 0.3 mg/kg of colchicine results in an 85% fall in microtubule content and only a 30% fall in VLDL-TG secretion. This difference is statistically significant ($P < 0.01$). In keeping with the idea that VLDL-TG secretion may be functionally related to microtubule content, one could interpret this observation to mean that microtubules are disrupted in advance of the deterioration of VLDL-TG secretion.

The disorganization of Golgi complexes observed in hepatocytes after colchicine treatment

TABLE I

Effect of Colchicine on Content of Hepatocyte Organelles

Agent	Animals*	Structures (volume density × 10^2)				
		Golgi	Lysosomes	VLDL (Golgi)	VLDL (lysosome)	VLDL (endoplasmic reticulum)
mg/kg body wt						
Saline	10	2.30 ± 0.71	0.74 ± 0.22	0.48 ± 0.14	0.06 ± 0.02	0.25 ± 0.06
Colchicine						
0.3	5	2.08 ± 0.60	1.88 ± 0.25‡	0.39 ± 0.10	0.41 ± 0.08‡	0.85 ± 0.15§
0.5	5	1.70 ± 0.75	—	—	—	—
0.7	10	0.37 ± 0.11‡	1.50 ± 0.26§	0.02 ± 0.02‡	0.42 ± 0.08‡	1.67 ± 0.20‡
1.0	5	0.39 ± 0.16§	—	—	—	—

* Figures in this column represent the numbers of animals from which tissue was obtained for morphometric analysis 5 h after the administration of saline or colchicine.

‡ $P < 0.01$ as compared to values obtained after saline treatment.

§ $P < 0.05$ as compared to values obtained after saline treatment.

may also be related to the loss of microtubules. 5 h after treatment of rats with 0.15 or 0.30 mg of colchicine/kg, the content and distribution of Golgi complexes appear quite normal. After 0.5 mg colchicine/kg, when microtubules are reduced to <10% of the control value, Golgi complexes show a small, yet statistically insignificant, fall in content. However, the content of intact Golgi complexes is reduced to 10% of the control value ($P < 0.01$) after the administration of 0.7 mg of colchicine/kg. At this point the remaining intact Golgi complexes are often associated with a few segments of microtubules, although microtubules are so generally scarce in hepatocytes from animals given this dose of colchicine that they cannot be measured at all in random cell samplings. When specifically quantitated, >95% of the few microtubules remaining in cells at this stage are closely associated with Golgi complexes. These observations suggest a special relationship between microtubules and components of the Golgi apparatus, which suggests that microtubules may be important in the maintenance of the Golgi complex. The fact that the administration of another antimicrotubule agent, vinblastine sulfate, leads to qualitatively similar changes lends support for this idea. This point has also been made by Patzelt et al. (10), using the parotid gland as a model.

The changes that occur in Golgi complexes after colchicine (or vinblastine) treatment take several forms. Clearly, dictyosome cisternal membranes are reduced in number after treatment with antimicrotubule agents. This observation has been made previously for Golgi complexes of cultured hepatocytes (3) and various other cell types (8, 9, 16). However, other components of Golgi complexes seem not to be altered by antimicrotubule agents and thus help in the identification of presumptive complexes. For example, altered Golgi complexes still include clusters of coated and smooth-surfaced vesicles in association with VLDL-filled vacuoles. Variable numbers of vacuoles contain electron-dense material and resemble lysosomes. In addition to these changes associated with Golgi regions, other changes in VLDL compartmentalization occur. Vacuoles containing VLDL accumulate throughout the cytoplasm and many reveal limiting membranes studded with ribosomes. In cells of control animals, <5% of the total VLDL found in cells are bounded by RER membranes: after colchicine (0.7 mg/kg), not only does the accumulated hepatocyte VLDL content increase, but 25% of the VLDL are now within rough-surfaced membranes.

Thus, as a result of treatment with antimicrotubule drugs, Golgi cisternal membranes disappear, and, perhaps as a consequence, VLDL accumulate within both rough and smooth-surfaced vacuoles. That the VLDL-filled vacuoles are not efficiently discharged is suggested by these and other (6, 17) studies of low VLDL-TG secretion rates after colchicine and/or vinblastine treatment. That the excess VLDL may be degraded by crinophagy is suggested by the increase of VLDL within electron-dense bodies after treatment with colchicine and/or vinblastine (see also references 6 and 17).

The morphometric techniques used in this study

permit us to localize and quantify aggregated (assembled) microtubule protein which is visible at the electron microscope level. However, submicroscopic aggregated states of microtubule protein may also exist in cells, and it is likely that antimicrotubule agents would cause the disaggregation of protein in this state, as well as that of visible microtubules. On the basis of this thinking and the specific findings of this study, it is possible to offer a working hypothesis to explain the role of microtubules (assembled microtubule protein) in hepatic VLDL secretion. Aggregated microtubule protein is essential in maintaining the structural integrity of Golgi membranes and associated structures. Antimicrotubule agents lead to a disaggregation of microtubule protein, and a substantial number of Golgi complexes become disorganized when the level of disaggregation reaches a critical point. It is this disorganization of the Golgi complexes which results in the accumulation of VLDL and impairment of VLDL secretion, possibly because the membranes of the formed VLDL-containing vacuoles lack a critical component for transport which is normally acquired in passage through the Golgi complex. Finally, it is the persistent association of some microtubule protein with Golgi complexes, and the resulting persistence of a small number of intact Golgi complexes, which permit the secretion of VLDL-TG to continue at a markedly reduced level. Alternatively, one could postulate that a minor portion of newly synthesized VLDL is normally discharged in a nonregulated (non-Golgi) pathway (4), and that this pathway continues to function despite treatment of animals with antimicrotubule agents.

Obviously, this hypothesis is speculative and requires experimental verification. Alternative explanations can be offered. For example, the lack of parallelism between microtubule volume density and VLDL secretion at 0.3 mg/kg colchicine could be interpreted to mean that no relationship exists between microtubules and VLDL secretion. Redman et al. (15), utilizing very high doses of colchicine (100 mg/kg) and very short periods of treatment (1 h) have, in fact, reported a dissociation between hepatocyte microtubule content and albumin release (albumin presumably being transported by the same intracellular structures as VLDL), and concluded that microtubules are not directly associated with albumin transport. However, it must be mentioned that microtubules were not measured in that study, and it is possible that a quantitative relationship between total microtubule content and albumin secretion was missed. Even so, parallel responses do not prove that two events are related, and it is certainly possible that colchicine has other effects which are responsible for the observed disorganization of Golgi complexes and/or the inhibition of VLDL secretion. For example, colchicine has been shown to affect nucleoside transport (7). Moreover, colchicine has been shown to bind to (5, 18), and to affect the mobility of, a variety of membrane components (20, 21), and a direct (nonmicrotubule) effect on Golgi and/or Golgi-derived membranes cannot be discounted.

On the other hand, in a recent report, Caron and Berlin (2) have shown that microtubule protein can be selectively adsorbed to artificial phospholipid vesicles inducing stacking and/or fusion of the liposomes into multilamellar structures. If microtubule protein were bound to, and caused the stacking of, cellular membranes in vivo (such as Golgi cisternal membranes), one could imagine that colchicine could dissociate this binding and cause the type of structural change of the Golgi complex described in this study.

In any event, the fact that a totally different antimicrotubule agent, vinblastine sulfate, mimics every aspect of the colchicine effect makes it difficult to ignore the idea that microtubules are linked to secretory processes in the liver. For this reason, our current hypothesis ties microtubules (assembled microtubule protein) to the various kinetic and morphological events observed after colchicine treatment, and describes a permissive action for microtubules in VLDL-TG secretion as postulated in an earlier publication (14).

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REFERENCES

1. BERGMEYER, H. U. 1974. Methods of Enzymatic Analysis. Academic Press, Inc., New York. 1831.
2. CARON, J. M., and R. D. BERLIN. 1979. Interaction of microtubule proteins with phospholipid vesicles. *J. Cell Biol.* **81**:665–671.
3. DEBRABANDER, M., J. C. WANSON, R. MOSSELMANS, G. GEUENS, and P. DROCHMANS. 1978. Effects of antitubular compounds on monolayer cultures of adult rat hepatocytes. *Biol. Cell.* **31**:127–140.

4. JONES, A. L., N. B. RUDERMAN, and M. GUILLERMO HERRERA. 1967. Electron microscopic and biochemical study of lipoprotein synthesis in the isolated perfused rat liver. *J. Lipid Res.* **8**:429–446.
5. KORNGUTH, S. E., and E. SUNDERLAND. 1975. Isolation and partial characterization of a tubulin-like protein from human and swine synaptosomal membranes. *Biochim. Biophys. Acta.* **393**:100–114
6. LE MARCHAND, Y., A. SINGH, F. ASSIMACOPOULOS-JEANNET, L. ORCI, C. ROUILLER, and B. JEANRENAUD. 1973. A role for the microtubule system in the release of very low density lipoproteins by perfused mouse liver. *J. Biol. Chem.* **248**:6862–6870.
7. MIZEL, S. B., and L. WILSON. 1972. Nucleoside transport in mammalian cells. Inhibition by colchicine. *Biochemistry.* **11**:2573–2578.
8. MOSKALEWSKI, S., J. THYBERG, S. LOHMANDER, and V. FRIBERG. 1975. Influence of colchicine and vinblastine on the Golgi complex and matrix deposition in chondrocyte aggregates. *Exp. Cell Res.* **95**:440–454.
9. MOSKALEWSKI, S., J. THYBERG, and V. FRIBERG. 1976. In vitro influences of colchicine on the Golgi complex in A- and B- cells of guinea pig pancreatic islets. *J. Ultrastruct. Res.* **54**:304–317.
10. PATZELT, C., D. BROWN, and B. JEANRENAUD. 1977. Inhibitory effect of colchicine on amylase secretion by rat parotid glands. Possible localization in the Golgi area. *J. Cell Biol.* **73**:578–593.
11. REAVEN, E. P. 1977. Quantitative analysis of tubulin and microtubule compartments in isolated rat hepatocytes. *J. Cell Biol.* **75**:731–742.
12. REAVEN, E. P., and G. M. REAVEN. 1977. The distribution and content of microtubules in relation to the transport of lipid. An ultrastructural quantitative study of the absorptive cell of the small intestine. *J. Cell Biol.* **75**:559–572.
13. REAVEN, E., R. MAFFLY, and A. TAYLOR. 1978. Evidence for involvement of microtubules in the action of vasopressin in toad urinary bladder. III. Morphological changes in the content and distribution of microtubules in the bladder epithelial cells. *J. Membr. Biol.* **40**:251–267.
14. REAVEN, E., and G. M. REAVEN. 1978. Dissociation between rate of hepatic lipoprotein secretion and hepatocyte microtubule content. *J. Cell Biol.* **77**:735–742.
15. REDMAN, C. M., D. BANERJEE, K. HOWELL, and G. E. PALADE. 1975. Colchicine inhibition of plasma protein release from rat hepatocytes. *J. Cell Biol.* **66**:42–59.
16. SEYBOLD, J., W. BIEGER, and H. F. KERN. 1975. Studies on intracellular transport of secretory proteins in the rat exocrine pancreas. *Virchows Arch. A Pathol. Anat. Histol.* **368**:309–327.
17. SINGH, A., Y. LE MARCHAND, L. ORCI, and B. JEANRENAUD. 1975. Colchicine administration to mice: a metabolic and ultrastructural study. *Eur. J. Clin. Invest.* **5**:495–505.
18. STADLER, J., and W. W. FRANKE. 1974. Characterization of the colchicine binding of membrane fractions from rat and mouse liver. *J. Cell Biol.* **60**:297–303.
19. WEIBEL, E. R. 1973. Stereological techniques for electron microscopic morphometry. *In* Principles and Techniques of Electron Microscopy. M. H. Hayat, editor. **3**:237–291.
20. WUNDERLICH, F., and R. MULLER. 1973. Direct evidence for a colchicine-induced impairment in the motility of membrane components. *Science (Wash. D. C.).* **182**:1136–1138.
21. YAHARA, I., and G. M. EDELMAN. 1975. Modulation of lymphocyte receptor mobility by concanavalin A and colchicine. *Ann. N. Y. Acad. Sci.* **253**:455–469.