A Role for the Microtubular System in the Release of Very Low Density Lipoproteins by Perfused Mouse Livers*

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SUMMARY

Normal mouse livers have been perfused in situ and the release of triglycerides in the perfusate has been measured as an index of very low density lipoprotein secretion. Vincristine or colchicine, drugs known to interfere with the microtubular system, were found to inhibit markedly triglyceride release by perfused livers. When used at appropriate concentrations these drugs did not change glucose production, ureogenesis, ATP levels, or oxygen consumption. The uptake and oxidation of fatty acids by livers perfused with either drugs remained unaffected. Labeled oleate incorporation into total triglycerides (i.e. liver plus perfusate) as well as that of labeled amino acids into total proteins were not changed by vincristine or colchicine. The ultrastructure of livers perfused with these drugs appeared normal except that the microtubules could no longer be observed. Concomitantly numerous clusters of vesicles were observed in vincristine- or colchicine-treated livers. In the presence of vincristine (not in that of colchicine) these vesicles were very often seen in close relationship with amorphous masses interpreted as being precipitated microtubular proteins. It is concluded that the functional integrity of microtubules is important for the intracellular movement and eventual release of very low density lipoprotein particles. The additional observation that total protein secretion by the liver was also markedly inhibited by vincristine or colchicine further suggests that microtubules may also play a role in the release of albumin or globulins.

The liver has been shown to be a major site of biosynthesis and release of very low density lipoproteins which are of particular interest since they are important metabolic fuels for several tissues (1-3). Study of the formation and release of VLDL particles has been helped by the recognition that these lipoproteins can be identified with the electron microscope as opaque particles of 300 to 1000 A in diameter (4-10). This finding has considerably aided the study of the time sequence of their formation and release (4-7).

It has been demonstrated that the secretion of several hormones stored as granules such as insulin (11, 12), thyroxine (13, 14), and catecholamines (15) involves, at least in part, the microtubules of the respective cells. As VLDLs are also particulate secretion products, we have hypothesized that their migration within hepatocytes and therefore their eventual release might similarly be mediated by the microtubular system. The drugs vincristine or colchicine are known to interfere with the microtubular system of several different cell types (16-21). These agents therefore provide a means of assessing the possible role of this system in VLDL release. In the present study, vincristine and colchicine have been shown to inhibit markedly the release of VLDLs by perfused mouse livers at a time when accumulation of VLDL-like particles within the hepatocytes is observed with the electron microscope. These findings suggest that the secretion of VLDLs does require a functioning microtubular system. Preliminary reports of this work have been published (22, 23).

MATERIALS AND METHODS

Animals—Six-week-old, normal male Swiss mice weighing between 29 and 32 g, fed ad libitum with Altromin “R” laboratory chow and bred in these laboratories have been used.

Perfusion Technique and Medium—Mice were anesthetized by intraperitoneal injection of pentobarbital (100 mg per kg body weight), and livers were perfused in situ according to a method described previously (24) with the following modifications. Livers were initially perfused for 2 to 3 min with oxygenated Krebs-Ringer bicarbonate buffer to remove mouse blood and to prevent intravascular clotting and consequent swelling which invariably occurred if this step was omitted. This buffer was discarded as it flowed from the sectional vena cava. Following this procedure, livers were perfused with 50 ml of a recirculating medium consisting of Krebs-Ringer bicarbonate buffer con-

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† The abbreviation used is: VLDL, very low density lipoproteins.
containing 20% bovine erythrocytes washed as previously described (24); 1.5% bovine serum albumin defatted according to Goodman (25) to which 1.4 to 1.8 mg oleate had been bound, and 25% bovine serum. Prior to use the bovine serum was treated with active charcoal (3.5 g/100 ml) at pH 3.3 for 2 hours at 4°C (26), then centrifuged and filtered through a Sartorius filter (0.15-μm pore size, Sartorius Company, Göttingen, Germany). The charcoal-free serum thus obtained was adjusted to pH 7.4 and dialyzed at 4°C for 72 hours against three changes of 10 volumes of Krebs-Ringer bicarbonate buffer to restore normal ionic composition. This procedure was necessary to prevent the sudden swelling that occurred if livers were perfused with untreated serum. Presumably charcoal treatment removes some coagulation factors from the bovine serum. [9,10-3H]-Oleate or unlabeled oleate was bound to albumin by dropwise addition of its sodium soap to a 14% solution of bovine serum albumin. Labeled oleate (40 μCi of specific activity 2000 mCi per mmole) and randomly labeled U-aminio acid mixture or algal protein hydrolysate (10 μCi of specific activity 45 mCi per gram nitrogen) were added to the 50 ml of perfusion medium immediately before use. Vincristine sulfate or colchicine was dissolved in isotonic saline and 0.5 ml added to the 50 ml of perfusion medium. With the three perfusion apparatuses available, one or two series of three control and three test livers were similarly processed.

Analysis of Perfusate and Tissues—Aliquots (3 to 4 ml) of the perfusion medium were taken at the beginning of the perfusion and at 30-min intervals; 0.5 ml was immediately deproteinized with 0.5 ml of 1 N perchloric acid and, following centrifugation, used for acetoacetate (27) and β-hydroxybutyrate determination (28). The remaining medium was centrifuged at 4°C and the supernatant used for the following measurements: 0.01 ml for glucose (29), 0.1 ml for urea (30), 0.2 ml for free fatty acids (31), and 0.20 ml for triglycerides. Because of the small size of mouse livers, and because the volume of perfusion medium was the same as that previously used in rat liver experiments, the perfusate triglyceride concentration was 5 to 10 times less than the values reported for rat livers (32, 33). For this reason triglycerides were measured following hydrolysis by determining fluorometrically their glycerol content according to the method of Wieland (34) adopted as follows: Aliquots of 0.2 ml of perfusate were added to 0.2 ml of distilled water, approximately 10 mg of zeolite and 10 volumes of chloroform-methanol (2:1), then carefully shaken and left overnight. Following extraction the two-phase system obtained was centrifuged, and 0.5-ml aliquots of the triglyceride containing lower phase were transferred to small tubes, dried under a stream of nitrogen, and saponified for 40 min at 75°C with 0.5 ml of 0.5 N alcoholic KOH containing 20% isopropyl alcohol. To these samples was added 0.5 ml of 0.3 M MgSO₄ to readjust their pH, and to precipitate most of the free fatty acids as magnesium salts since their presence was found to interfere with the enzymatic measurement of glycerol. Following centrifugation, these samples were transferred to new tubes containing 0.8 ml of chloroform and then washed again with 5% trichloroacetic acid following the method of Schneider (38). The final precipitate thus obtained was dissolved in 1 N NaOH and 1 aliquot of it counted for its radioactive content, while another was analyzed for its protein content according to Lowry (39) with bovine albumin as a standard. The presence of vincristine or colchicine did not alter any of the measurements made.

Electron Microscopic Studies—Livers were fixed by perfusion at room temperature for 10 min with 2% phosphate-buffered glutaraldehyde. Small blocks were then postfixed for 2 hours with cold 2% phosphate-buffered osmium tetroxide, dehydrated in graded ethanol and embedded in Epon mixture (Epikote, Shell Company). The thin sections were sequentially stained with uranyl acetate and lead citrate and examined in a Philips EM300 microscope.

Chemicals—All organic and inorganic chemicals were purchased from E. Merck (Darmstadt, Germany) or Fluka A. G. (Buchs, Switzerland) and were of analytical grade. Labeled compounds came from the Radiochemical Centre (Amersham, Buckinghamshire, England). Most enzymes and coenzymes used in these studies were a gift of Dr. Schmidt (Boehringer Mannheim GmbH, Mannheim, Germany). Firefly lantern extracts came from Sigma Chemical Company (St. Louis, Missouri). Colchicine was purchased from E. Merck. Vincristine sulfate (Oncovin) was a gift of Eli Lilly Laboratories (Indianapolis, Indiana). Amino acid mixture (TC amino acids Hela 100 X) was obtained from Difco Laboratories (Detroit, Michigan). Zeolite was secured from Serva (EntwicklungsLabor, Heidelberg, Germany).
RESULTS

Effects of Vincristine or Colchicine on Triglyceride Secretion—Since VLDL triglycerides accounted for about 80% (control livers 77% ± 2; "mitotic-spindle" inhibitor-treated livers 89% ± 10) of total perfusate triglycerides, net triglyceride output has been taken as an index of liver VLDL secretion. As illustrated in Fig. 1, the triglyceride output of control livers was about 8 µmoles/100 g body weight per hour for the first 30 min of perfusion, after which it increased to 12 to 15 µmoles/100 g per hour and remained linear for the next 134 hours. In the presence of rather high concentrations of either vincristine (2.5 µM or colchicine 85 µM), triglyceride output was markedly depressed. The inhibitory effect was evident within 30 min, and became more marked with increasing duration of perfusion. During the second hour, triglyceride secretion by livers treated with vincristine or colchicine reached the very low rate of 1.5 to 3 µmoles/100 g per hour, and after 2 hours of perfusion with either of these agents, net perfusate triglyceride concentration was only 25 to 30% that of controls. The inhibitory effect of vincristine or colchicine could be obtained at lower concentrations, as shown in Table I. Vincristine at 0.4 µM was somewhat inhibitory after only 60 min of perfusion but became markedly so after 2 hours, at which time perfusate triglyceride concentration was half that of control livers. No inhibitory effect of vincristine could be observed when used at the concentration of 0.04 µM. Colchicine used at the concentration of 10 µM was as effective as that of 85 µM (Fig. 1, Table I) and still effective at 1 µM, a concentration which reduced perfusate triglyceride concentration to half that of control livers after 2 hours of perfusion. At 0.1 µM colchicine was no longer inhibitory. It is of interest that the combined addition of colchicine and vincristine.

Fig. 1. Effect of vincristine or colchicine on triglyceride secretion by the perfused mouse liver. Livers from fed mice were perfused with 50 ml of recirculating Krebs-Ringer bicarbonate buffer containing 20% washed bovine erythrocytes, 1.5% bovine albumin containing 1.4 to 1.8 mM oleate, 25% bovine charcoal-treated serum. Controls, △—△ vincristine, O—.-.O (2.5 µM), or colchicine, ●—● (80 µM) was added at time 0. Each point is the mean of 12 (controls) or 6 (vincristine, colchicine) values ± S.E.M.

Table I

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration (µM)</th>
<th>No of</th>
<th>Triglyceride output after</th>
<th>Triglyceride output after</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>exp.</td>
<td>(µmoles / 100 g body weight)</td>
<td>(µmoles / 100 g body weight)</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>4</td>
<td>3.1 ± 0.5</td>
<td>9.7 ± 0.9</td>
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<tr>
<td>Vincristine</td>
<td>0.4</td>
<td>4</td>
<td>3.6 ± 0.4</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.04</td>
<td>4</td>
<td>3.9 ± 0.1</td>
<td>10.2 ± 0.6</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>4</td>
<td>4.8 ± 0.5</td>
<td>13.0 ± 0.6</td>
</tr>
<tr>
<td>Colchicine</td>
<td>1</td>
<td>4</td>
<td>7.1 ± 0.6</td>
<td>7.1 ± 0.6</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>4</td>
<td>5.0 ± 1.5</td>
<td>13.0 ± 1.7</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.1</td>
<td>3</td>
<td>4.3 ± 0.6</td>
<td>9.2 ± 1.3</td>
</tr>
<tr>
<td>Colchicine</td>
<td>0.04</td>
<td>2</td>
<td>7.3 ± 0.6</td>
<td>16.5 ± 1.5</td>
</tr>
<tr>
<td>None</td>
<td>-</td>
<td>4</td>
<td>2.8 ± 0.5</td>
<td>11.4 ± 1.7</td>
</tr>
<tr>
<td>Vincristine</td>
<td>0.04</td>
<td>3</td>
<td>7.6 ± 1.2</td>
<td>9.7 ± 2.0</td>
</tr>
</tbody>
</table>

* Significantly different from controls at p < 0.05.
** Significantly different from controls at p < 0.01.
*** Significantly different from controls at p < 0.005.
at concentrations that were ineffective when used singly resulted in a significant inhibitory effect with a total triglyceride output only 30% of control values after 2 hours of perfusion.

**Further Biochemical Studies**—The above mentioned inhibition of triglyceride output by vincristine or colchicine could conceivably be due to nonspecific toxic effects of these drugs on the liver. To rule out this possibility, some basic functions of the liver, e.g. glucose production, ureogenesis, ATP levels, and oxygen consumption were measured in both control and treated livers. As illustrated in Table II, only one of these metabolic indices, the production of urea, was inhibited (20%) by colchicine (85 μM), an effect that was no longer present when the concentration of the drug was reduced to 10 μM. Although not shown in Table II, when urea output was increased by the addition of amino acids to the perfusion medium, it was similarly unaffected by the presence of 10 μM colchicine. For these reasons, the latter concentration of colchicine was used in all subsequent experiments.

A reduction in triglyceride output by livers treated with vincristine or colchicine could have been the result of a decrease in the uptake of oleate from the perfusion medium or an increase in the oxidation of oleate once taken up by the hepatocytes. That this was not the case is shown by the results of Table III which demonstrate that neither vincristine nor colchicine modified fatty acid uptake or fatty acid oxidation to ketone bodies at any of the perfusion times tested. As the observed decrease in triglyceride-rich lipoprotein output produced by vincristine or colchicine could have been caused by decreased lipogenesis or decreased protein synthesis, experiments were carried out in which labeled oleate incorporation into hepatic and perfusate triglycerides, as well as labeled amino acid incorporation into hepatic and perfusate proteins was measured. The results of these experiments are summarized in Table IV. It can be seen that not only was oleate incorporation into hepatic triglycerides not decreased by vincristine or colchicine but actually increased by both agents. Although this increase did not reach the level of statistical significance for vincristine it was highly significant.

**Table II**

**Lack of effect of vincristine or colchicine on glucose production, ureogenesis, cellular ATP, and oxygen consumption in perfused mouse livers**

Livers were perfused for 2 hours as described in Fig. 1. ΔpO₂ was obtained by measuring pO₂ in the perfusate entering and leaving the liver. Figures are presented as means ±S.E.M.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration (μM)</th>
<th>% of exp</th>
<th>Glucose production (μmoles/100 g body weight)</th>
<th>Urea production (μmoles/100 g body weight)</th>
<th>Cellular ATP content (μmoles/100 g wet liver weight)</th>
<th>ΔpO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>2.5</td>
<td>6</td>
<td>694 ± 103</td>
<td>106 ± 9</td>
<td>1.76 ± 0.11</td>
<td>172 ± 17</td>
</tr>
<tr>
<td>Vincristine</td>
<td>85</td>
<td>6</td>
<td>624 ± 101</td>
<td>104 ± 9</td>
<td>1.87 ± 0.15</td>
<td>175 ± 19</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>4</td>
<td>706 ± 27</td>
<td>113 ± 10</td>
<td>1.84 ± 0.21</td>
<td>---</td>
</tr>
<tr>
<td>Colchicine</td>
<td>10</td>
<td>4</td>
<td>752 ± 52</td>
<td>111 ± 8</td>
<td>1.96 ± 0.08</td>
<td>---</td>
</tr>
</tbody>
</table>

* Significantly different from controls at p < 0.05.

**Table III**

**Lack of effect of vincristine or colchicine on fatty acid uptake and oxidation by perfused mouse livers**

Livers were perfused for 120 min as described in Fig. 1. Ketone body production was the sum of acetoacetate plus β-hydroxybutyrate. Figures are presented as means ± S.E.M. All differences from controls were not significant.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration (μM)</th>
<th>% of exp</th>
<th>Fatty acid uptake after: (μmoles/100 g body weight)</th>
<th>Fatty acid oxidation to Ketone bodies after: (μmoles/100 g body weight)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>6</td>
<td>80 ± 6</td>
<td>131 ± 9</td>
</tr>
<tr>
<td>Vincristine</td>
<td></td>
<td>6</td>
<td>77 ± 4</td>
<td>117 ± 7</td>
</tr>
<tr>
<td>None</td>
<td>2.5</td>
<td>6</td>
<td>81 ± 4</td>
<td>132 ± 5</td>
</tr>
<tr>
<td>Colchicine</td>
<td>85</td>
<td>6</td>
<td>61 ± 4</td>
<td>147 ± 6</td>
</tr>
<tr>
<td>None</td>
<td>10</td>
<td>4</td>
<td>71 ± 8</td>
<td>165 ± 8</td>
</tr>
<tr>
<td>Colchicine</td>
<td></td>
<td>4</td>
<td>77 ± 8</td>
<td>141 ± 7</td>
</tr>
</tbody>
</table>
TABLE IV

Effect of vincristine or colchicine on labeled oleate incorporation into triglycerides or amino acid into proteins by perfused mouse livers

Livers were perfused for 2 hours with the same medium as described in Fig. 1 but containing initially 40 μCi of [9,10H]oleate or 10 mg of amino acid mixture plus 10 μCi of 14C uniformly labeled amino acids. Figures are presented as means ± S.E.M.

<table>
<thead>
<tr>
<th>Additions</th>
<th>Concentration (μM)</th>
<th>N° of exp.</th>
<th>Incorporation of labeled oleate (dpm/mouse/2hr x 10^-5)</th>
<th>Incorporation of labeled amino acid (dpm/mouse/2hr x 10^-5)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>hepatic triglycerides</td>
<td>perfusate triglycerides</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>4</td>
<td>14.94 ± 1.11</td>
<td>11.78 ± 0.92</td>
</tr>
<tr>
<td>Vincristine</td>
<td>2.5</td>
<td>6</td>
<td>20.96 ± 2.84</td>
<td>3.60 ± 0.51</td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>6</td>
<td>13.34 ± 0.55</td>
<td>19.07 ± 1.87</td>
</tr>
<tr>
<td>Colchicine</td>
<td>10</td>
<td>3</td>
<td>38.2 ± 1.99</td>
<td>6.16 ± 0.66</td>
</tr>
</tbody>
</table>

* Not significantly different from controls.
** Significantly different from controls at p < 0.025.
*** Significantly different from controls at p < 0.005.
**** Significantly different from controls at p < 0.0005.

for colchicine. Secretion of labeled triglycerides was, as expected from the preceding results, markedly decreased by these drugs and, more importantly, total oleate incorporation into liver plus perfusate triglycerides was similar in control and vincristine-treated livers, and only slightly smaller in colchicine-treated livers. Though not shown in Table IV, amino acid incorporation into liver triglycerides was also higher in vincristine- or colchicine-treated livers than in control livers (controls: 1.17 ± 0.82, 10 μM vincristine: 1.7 ± 0.54, 10 μM colchicine: 1.6 ± 0.45, not significant). Furthermore, the unlabeled triglyceride accumulation measured in each liver by the difference between triglyceride content before and after perfusion by means of an initial biopsy was higher in livers treated with 2.5 μM vincristine (2.45 mmoles per g wet liver ± 0.35) or 10 μM colchicine (4.77 ± 0.34) than in controls (0.23 ± 0.05).

Table IV also shows that the incorporation of labeled amino acids into hepatic proteins tended to be higher in vincristine- or colchicine-treated, than in control livers. Similarly, labeled amino acid incorporation into perfusate proteins was lower in vincristine- or colchicine-treated livers, and the incorporation of amino acids into total proteins of liver plus perfusate was not smaller but actually somewhat greater in the livers treated with the inhibitors than in control livers. The dynamics of the effect of colchicine upon incorporation of labeled oleate into labeled triglycerides is shown in Fig. 2A. Although not shown in Fig. 2, identical results were obtained when vincristine was used instead of colchicine. It can be seen that following an initial period of 30 min, during which less than 3% of the total triglyceride release occurred, the output of triglyceride was linear in both control and colchicine-treated livers although it was markedly inhibited in the latter case. Finally, it should be stressed (Fig. 2B) that labeled amino acid incorporation into total trichloroacetic acid-
precipitable proteins released into the perfusate was lower in colchicine-treated than in control livers. Similar observations were made with vincristine-treated livers.

**Electron Microscopic Studies**—Livers perfused for 2 hours with or without vincristine or colchicine always showed a very highly satisfactory state of preservation as judged by the ultrastructural appearance of cell organelles. Although quantitative data are not yet available, fat droplets occurring free within the cytoplasm were seen with about the same frequency in both control and treated livers. In control livers electron-opaque particles of 300 to 1000 Å in diameter could be observed, as previously reported (4-6), in the Golgi complex, in membrane-bound vesicles within the cytoplasm, and free in the space of Disse (Fig. 3). They were likely to represent, as proposed by other investigators (4-6), triglyceride-rich VLDL particles. Prominent changes were observed in livers that had been perfused with vincristine, as illustrated in Fig. 4. The most striking finding was the accumulation, never observed in control livers, of a considerable number of vesicles containing many electron-opaque particles of 300 to 1000 Å diameter. These particles appeared morphologically similar to the VLDL particles observed in control livers (Fig. 3, References 4-6) and will be subsequently referred to as VLDL-like particles. The vesicles containing the VLDL-like particles were commonly associated in clusters of 3 to 15 and could be observed anywhere within the cytoplasm but more often close to the vascular pole. An additional striking feature (Fig. 4) was the observation that the space of Disse of vincristine-treated livers was almost completely devoid of the VLDL-like particles commonly observed in this site in control livers. In control livers, microtubules (Fig. 3) could be found scattered throughout the cytoplasm as previously described (40). In contrast, vincristine-treated livers were virtually devoid of these organelles. The disappearance of microtubules was paralleled by the accumulation of apparently amorphous masses that were usually seen in close relationship with the vesicles containing VLDL-like particles (Fig. 5). The typical vincristine-induced crystals reported for other tissues (12, 16) were observed in perfused livers only with high, toxic concentrations of the drug or with lower concentrations, only within the Kupffer cells. Following perfusion of livers with colchicine (Fig. 6), a similar accumulation of vesicles containing VLDL-like particles was noted as well as marked reduction of such particles in the space of Disse. Concomitantly, microtubules were almost completely lacking but the amorphous masses noted in vincristine-treated livers were not observed.

**Discussion**

The striking finding of the present study is that “mitotic-spindle inhibitors” (vincristine, colchicine) cause a marked decrease in net triglyceride secretion by the perfused mouse liver. Since the triglycerides produced by the perfused liver are secreted primarily in the form of very low density lipoproteins, it is proposed that the microtubular-microfilamentous system of liver cells plays a role in the intracellular movement and eventual release of very low density lipoproteins, and might prove to be an

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With regard to the criteria used to name these particles VLDL-like particles, one should mention that these particles do have similar electron microscopic appearance and diameter as the VLDL particles previously described (4). Although it is thought that they are likely to be VLDL particles, there is as yet no proof for it, hence the name “VLDL-like” particles.

The lack of microtubules in vincristine-treated livers was conspicuous. However, no biochemical widening and no serial sections are available yet to definitely prove their total lack.

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Fig. 3. A, vascular pole of an hepatocyte from control liver. Numerous VLDL-like particles (large arrows) are seen within Disse's space. Cytoplasmic microtubules, which are randomly arranged, have been delineated by a series of small arrows. Gl, glycogen; Mv, microvilli; E, endothelium. × 20,000. B, portion of an hepatocyte depicting microtubules in longitudinal (series of small arrows) and cross (large arrow) sections. Gl, glycogen; M, mitochondrion. × 45,000
FIG. 4. A, portions of hepatocytes from vincristine-treated (2.5 μm) liver. Collections of vesicles containing VLDL-like particles are indicated by the arrows. The space of Disse (D) appears devoid of such particles. E, endothelium; Gl, glycogen; L, lipid; M, mitochondrion; RER, rough endoplasmic reticulum; AV, autophagic vacuoles. × 16,000. B, vascular pole of an hepatocyte from the same liver as shown in A. The space of Disse (D) is devoid of VLDL-like particles. E, endothelium; Mv, microvilli. × 30,000.
FIG. 5 (left). Portions of hepatocytes from liver treated with vincristine (2.5 \, \text{\mu}M). \( A \), several masses of amorphous material (asterisks) are seen in the proximity of vesicles containing VLDL-like particles (v). \( \times 49,000 \). \( B \), the asterisks indicate amorphous material in close relationship with a cluster of vesicles containing VLDL-like particles (v). \( \times 42,000 \).

FIG. 6 (right). Portion of hepatocyte from liver treated with colchicine (85 \, \text{\mu}M). The arrows indicate two collections of vesicles containing VLDL-like particles of varying electron opacity. RER, rough endoplasmic reticulum; M, mitochondrion. \( \times 26,000 \).

important site of regulation. The inhibitory effect of vincristine (2.5 \, \text{\mu}M) or colchicine (10 or 85 \, \text{\mu}M) must be very rapid since no lag period was observed (Figs. 1 and 2). The residual secretion of triglycerides measured in such treated livers may be due to the fact that all microtubules were not disrupted by the concentrations used. Total disruption of these organelles could not be assessed since higher concentrations of these agents produced nonspecific effects such as swelling of the rough surfaced endoplasmic reticulum, decreased ketogenesis and ureogenesis. When the concentration of vincristine or colchicine was reduced, the inhibitory effect on triglyceride release was of about the same magnitude but delayed (Table I). Further reduction in the concentration of these agents resulted in a loss of the inhibitory effect (Table I). The observed decrease in triglyceride release brought about by the combination of vincristine and colchicine used at concentrations that were ineffective when each drug was used alone suggests that these agents may be additive (Table I).

Although the present experiments do not prove that the effect of vincristine or colchicine on triglyceride secretion is the result of disruption of microtubules, such a mechanism is strongly suggested by the fact that, at the concentrations employed, no deleterious effects other than disruption of the microtubules could be detected as discussed below. Electron microscopic studies revealed that the state of preservation of vincristine- or colchicine-treated livers was satisfactory but that the microtubules had almost completely disappeared (Figs. 4 to 6). In vincristine- but not in colchicine-treated livers masses of amorphous material were seen in the cytoplasm. These masses are tentatively interpreted as being the result of precipitation of microtubular proteins by vincristine. The lack of such masses in colchicine-treated livers may be due to the fact that colchicine depolymerizes rather than precipitates microtubules. The most striking morphological finding observed in mitotic-spindle inhibitor-treated livers was the accumulation of VLDL-like particles enclosed within vesicles (Figs. 4 to 6). Such accumulation of VLDL-like particles was consistent with the existence of an at least normal production of these lipoproteins associated with a "paralysis" of their intracellular movement and release possibly due to altered microtubular function. The lack of VLDL-like particles in the space of Disse of treated livers strengthens this viewpoint.

Besides alteration in microtubular activity, triglyceride-rich lipoprotein output could conceivably be reduced by vincristine or colchicine either via unspecific, deleterious effects or via inhibitory effects on fatty acid uptake, on over-all lipogenesis or very low density lipoprotein-triglyceride synthesis, on over-
all protein or VLDL-protein synthesis. The results of the present experiments have excluded several of these possibilities. Thus, basic function of the liver (Table I) as well as fatty acid uptake or oxidation (Table III) were unaltered by the inhibitors. Total incorporation of labeled oleate into triglycerides was not changed by vincristine and only slightly decreased by colchicine (Table IV), indicating that fatty acid esterification proceeded normally in livers treated with either agents. Other experiments indicated that these agents did not interfere with the conversion of substrates such as amino acids to acetyl-CoA and fatty acids. Finally, the observed accumulation of triglycerides in livers treated with “spindle” agents strengthens the concept of a continuing lipogenesis despite blockade of lipid secretion.

It has been demonstrated previously that the inhibition of protein synthesis by cycloheximide (41), tetracycline (42), or paromomycin (4) resulted in a marked decrease in the secretion of triglycerides by the liver. However, in the present experiments, total incorporation of labeled amino acids into proteins was at least equal in “spindle agent”-treated than in control livers (Table IV). The synthesis of the protein moiety of VLDLs was not specifically measured and conceivably could have been inhibited by vincristine or colchicine. Although it cannot be ruled out, such a mechanism is unlikely since it has been proposed that once visible at the electron microscope very low density lipoproteins are fully synthesized (4, 7), and since there is an apparent increase in VLDL-like particles in livers treated with vincristine or colchicine. However, more definite conclusions concerning possible effects of vincristine or colchicine on the protein and lipid moieties of VLDLs must await the isolation of VLDLs from Golgi complexes. The additional observation that the secretion of total proteins by perfused livers treated by colchicine or vincristine suggests that microtubules may play a role not only in lipoprotein secretion but also in the release of proteins such as albumin or globulins (Fig. 2). This is in keeping with recent studies (43, 44) showing that albumin might be segregated together with VLDLs in the Golgi apparatus within vesicles which then move toward the vascular pole to release their content extracellularly. Finally, one should stress that the effect of “mitotic-spindle” inhibitors may be valid only under the experimental conditions used, and that microtubules could conceivably be modulated in a different way under different circumstances.

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