INHIBITION BY COLCHICINE OF FIBRINOGEN TRANSLOCATION IN HEPATOCYTES

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Colchicine inhibits the secretion into plasma of lipoproteins (12, 18), albumin (11), and other proteins (11) produced by the liver. Electron microscopy has shown that, after administration of colchicine, the electron-dense, nonsecreted lipoproteins accumulate in cytoplasmic vesicles (12) and also in the Golgi apparatus (18) of hepatocytes; however, the accumulation site of the electron-transparent, nonsecreted proteins is unknown. In the present report, we demonstrate that fibrinogen, an electron-transparent protein synthetized by the liver cells (16), accumulates not only in the Golgi apparatus, but also within the endoplasmic reticulum when its secretion into plasma is inhibited by colchicine.

Fibrinogen was demonstrated in the hepatocytes by means of antirat fibrinogen antibodies labeled with peroxidase, a method which can be used to detect antigens under light and electron microscopy (3).

MATERIALS AND METHODS

Adult male rats were injected with colchicine, 2.5 mg/kg, intraperitoneally, and were sacrificed 4, 6, 8, 16, 24, or 32 h after injection; rats, injected with 0.9% NaCl or with 2.5 mg/kg of lumicolchicine (a mixture of colchicine isomers prepared by irradiation of colchicine with ultraviolet light [20]) and sacrificed at the same time intervals, acted as controls; the experimental groups corresponding to each time interval consisted of five animals.

Antirat fibrinogen antibodies were prepared as follows: a sheep was immunized by an intradermic injection of 7 mg of rat fibrinogen added with Freund's complete adjuvant, followed by an intramuscular injection of fibrinogen with Freund's adjuvant every month for 4 mo; rat fibrinogen was prepared according to the method of Polson et al. (14). Antibodies against antigens other than fibrinogen were eliminated by an immunoadsorbent technique (4) using an immunoadsorbent prepared from normal rat serum; purified antirat fibrinogen antibodies were finally obtained by the same technique, using an immunoadsorbent prepared from normal rat plasma. From those purified antibodies, Fab fragments were prepared by digestion with 1% papain (15) and purified by filtration on a diethylaminoethyl-cellulose column (DE 52, Whatman Biochemicals Ltd., Maidstone, England) with sodium phosphate buffer 0.01 M pH 8.0; the Fab fragments were concentrated and labeled with horseradish peroxidase (RZ 3.0, Sigma Chemical Co., St. Louis, Mo.) according to Avrameas (2). For control reactions, Fab fragments were also prepared from normal sheep gamma globulins obtained by ammonium sulfate precipitation followed by filtration on a diethylaminoethyl-cellulose column (DE 52) with sodium phosphate buffer 0.017 M pH 6.9; those Fab fragments were also concentrated and labeled with horseradish peroxidase.

Liver specimens were processed according to a technique previously described (6). Briefly, the specimens were at once fixed in 10% paraformaldehyde (prepared as described by Karnovsky [10]) buffered with phosphate buffer 0.1 M pH 7.4 for 6 h at 4°C. After fixation, the liver pieces were washed for 24–48 h at 4°C in phosphate buffer 0.1 M pH 7.4. 8-μm thick sections cut with a cryostat were incubated for 1 h at room temperature with the labeled antibodies. After incubation, the sections were washed, and the peroxidase was demonstrated by the technique of Graham and Karnovsky (8). The sections were then placed on a thin plate of Epon (Shell Chemical Co., New York), fixed in a 1.5% osmium tetroxide solution buffered with Veronal buffer pH 7.2 for 20 min, embedded in Epon by the technique of

Zeitoun and Lehy (21) and studied in a light microscope without additional staining. Ultrathin sections were made at the levels of the cells in which a reaction had been noted in light microscopy and examined without further staining in a Siemens Elmiskop IA electron microscope. Control specimens were prepared by incubating sections with normal sheep Fab fragments labeled with peroxidase.

Conventional electron microscopy was also performed on the liver specimens. Small blocks were fixed in 2% glutaraldehyde buffered with phosphate buffer 0.1 M pH 7.4 for 2 h at 20°C, then postfixed in osmium tetroxide, and embedded in Epon. Ultrathin sections stained with uranyl acetate and lead citrate were observed by electron microscopy.

RESULTS

By optical microscopy in the controls at all time intervals, few hepatocytes containing fibrinogen were seen; they were randomly distributed within the hepatic lobule (Fig. 1). In colchicine-treated rats, 4 h and 6 h after the intraperitoneal injection of the drug, the findings were similar to those observed in the controls; at 8 h, numerous hepatocytes, with perinuclear, intensely brown deposits, were seen, mainly in the neighborhood of the portal triads (Fig. 2); at 16 h, numerous hepatocytes containing fibrinogen, but with a less intense reaction, were present predominantly in the vicinity of the portal triads; at 24 h, only a few hepatocytes with a slight coloration were present; and finally, at 32 h, the picture was similar to that of controls. In both colchicine-treated rats and controls, reactions with normal sheep Fab fragments were always negative.

By electron microscopy in the controls at all time intervals, fibrinogen was demonstrated exclusively on the ribosomes and membranes of the rough endoplasmic reticulum (*RER*) (Fig. 3) and on the membranes of the smooth endoplasmic reticulum (*SER*) of the hepatocytes; no fibrinogen was detected in the lumina of the endoplasmic reticulum and on the Golgi apparatus (*GA*). In colchicine-treated rats, 4 h and 6 h after the intraperitoneal injection of the drug, the findings were similar to those observed in the controls; at 8 h, fibrinogen was detected on the ribosomes and membranes of the *RER*; it was abundant in the lumina of this organelle (Fig. 4); it was detected in small amounts in the lumina of the *SER* and was absent in the *GA*; at 16 h, fibrinogen was detected in small amounts in the lumina of the *RER*; it was very abundant in the lumina of the *SER* and was absent in the *GA* (Fig. 5); at 24 h, fibrinogen was not detected in the lumina of the *RER*; it was detected in small amounts in the lumina of the *SER* and was abundant in the lumina of the *GA* (Fig. 6); at 32 h, the picture was similar to that of controls. In both colchicine-treated rats and controls, reactions with normal sheep Fab fragments were always negative.

By conventional electron microscopy in the controls, microtubules could be seen scattered throughout the cytoplasm of the hepatocytes. In colchicine-treated rats, at 4, 6, 8, 16, and 24 h after administration of the drug, microtubules were almost completely lacking; at 8 h, lysosome-like bodies were more numerous than in the controls; at 32 h, the appearance of the hepatocytes did not differ from that of the controls.

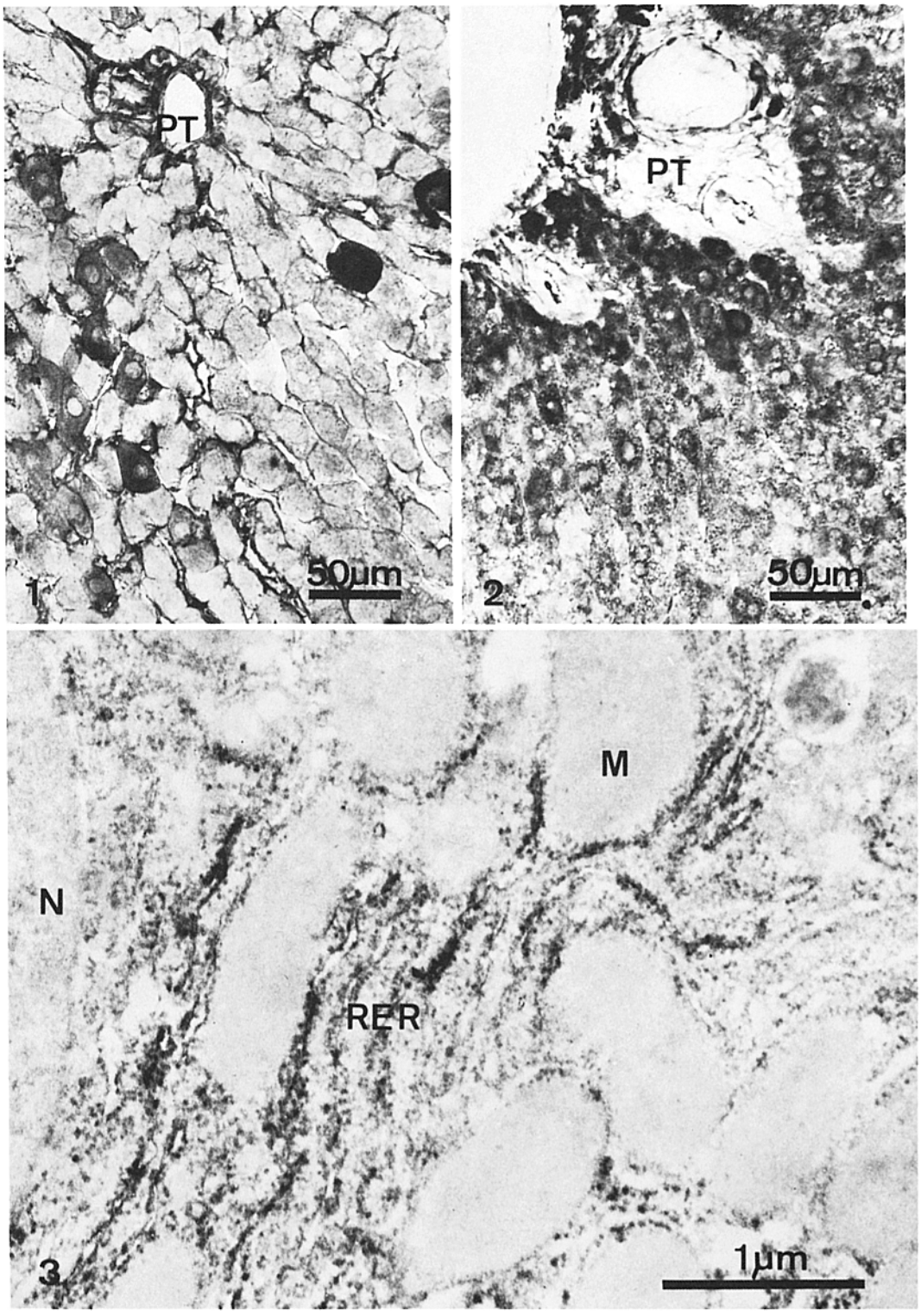
DISCUSSION

The small number of fibrinogen-containing hepatocytes observed by optical microscopy in normal rats is in agreement with similar findings obtained with immunofluorescence in man (9) and in the dog (5). The marked increase in the number of fibrinogen-containing hepatocytes observed in rats after administration of colchicine would be the result not of an increased synthesis of this protein but of its accumulation in the liver cells: this hypothetical interpretation is in line with the view

FIGURE 1 Light microscope appearance of the liver in a control rat. A dark deposit indicating the presence of fibrinogen is seen in the cytoplasm of few hepatocytes (*PT*, portal triad). × 275.

FIGURE 2 Light microscope appearance of the liver in a colchicine-treated rat 8 h after intraperitoneal injection of the drug. Numerous hepatocytes with perinuclear, intensely dark deposits indicating the presence of fibrinogen are seen in the neighborhood of the portal triad (*PT*). × 275.

FIGURE 3 Electron microscope appearance of a part of the rough endoplasmic reticulum (*RER*) of a hepatocyte from a control rat. Fibrinogen is only detected on the ribosomes and the membranes of the *RER*; no fibrinogen is detected in the lumina of this organelle. (*N*, nucleus; *M*, mitochondria.) × 31,000.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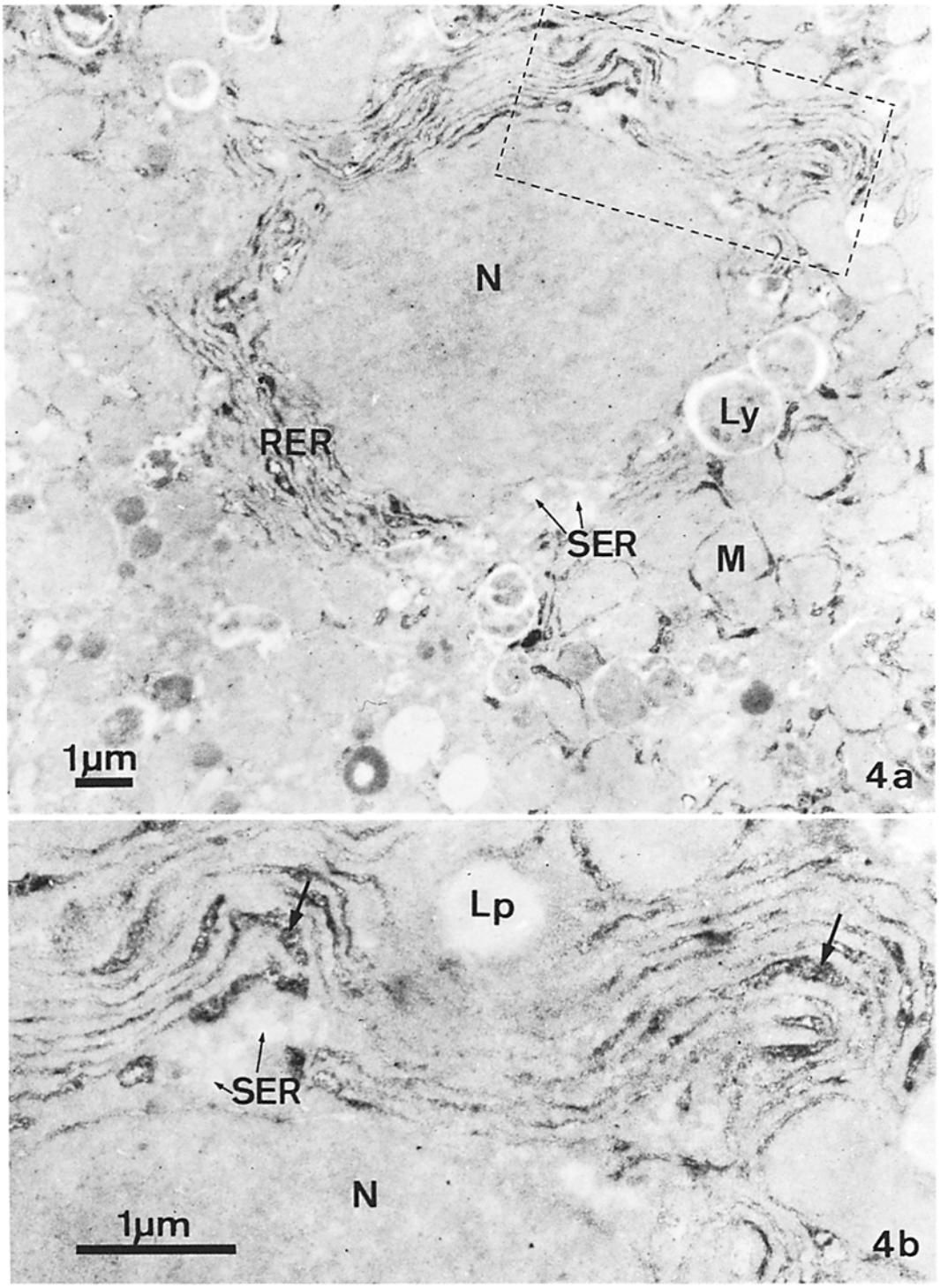

FIGURE 4 Electron microscope appearance of a hepatocyte from a colchicine-treated rat 8 h after intraperitoneal injection of the drug. (*a*) Fibrinogen is present in abundant amounts in several segments of the rough endoplasmic reticulum (*RER*) and is not detected in most of the vesicles of the smooth endoplasmic reticulum (*SER*). (*N*, nucleus; *M*, mitochondria; *Ly*, lysosome-like bodies.) × 8,500. (*b*) This figure is an enlargement of the area enclosed in the rectangle in Fig. 4 *a*. Fibrinogen is detected on the membranes and in the lumina (arrows) of the rough endoplasmic reticulum; fibrinogen is not detected in the smooth endoplasmic reticulum (*SER*). (*N*, nucleus; *Lp*, lipid droplet.) × 24,000.

that colchicine inhibits not the synthesis (11, 12, 18) but the secretion of proteins produced by the liver (11, 12, 18). The predominance of fibrinogen-containing hepatocytes in the vicinity of the portal triads might be due to the relatively high concentration of the drug in periportal hepatocytes since the drug reaches the liver through the portal vein.

The electron microscope observation that, early after colchicine administration, fibrinogen accumulates in the lumina of the *RER* suggests that the drug inhibits the translocation of fibrinogen through this organelle. That, late after colchicine administration, a bulk of fibrinogen appears in the lumina of the *SER*, and then in the *GA*, could be attributed to the gradual cessation of the inhibitory effect of the drug on the *RER* and/or an inhibition of fibrinogen translocation through the *SER* and the *GA;* this speculation is based on the current concept that the *RER*, the *SER*, and the *GA* represent the successive portions of a canalicular network through which the secreted proteins are conveyed (1, 7). That no fibrinogen was detected in the *GA* in control rats might be ascribed, not to its absence in the organelle, but to its concentration being too low to permit its detection by the method used in this work.

Our observations and our interpretation of these effects of colchicine are at variance with the findings of Stein et al. (18) who have showed that, early after administration of this drug, lipoproteins accumulate in *GA* vesicles and that colchicine inhibits the final stages of the secretion into plasma of lipoproteins; this discrepancy could be due to a different effect of colchicine on fibrinogen and lipoproteins; this interpretation is reinforced by the observation that, 4 h after administration of the drug, a detectable effect of colchicine was obtained on lipoprotein translocation, but not on fibrinogen translocation.

The action of colchicine on the cytoplasmic translocation of fibrinogen could be due to a direct effect of the drug on the membranes of the endoplasmic reticulum (17). Alternatively, it could be the indirect result of the disruptive effect of the drug on the microtubules (13, 19), an effect which has been observed in the hepatocytes from rats receiving colchicine by us and others (18) and in the hepatocytes from isolated mouse livers perfused with a medium containing colchicine (12); it is conceivable that microtubules, owing to their rigidity, are essential to maintain the suitable spatial conformation of the cytoplasmic canalicular network.

SUMMARY

In the rat, 8 h after intraperitoneal administration of colchicine, fibrinogen (detected by antirat fibrinogen antibodies labeled with peroxidase) accumulated in the lumina of the rough endoplasmic reticulum of the hepatocytes; 16 and 24 h after colchicine administration, fibrinogen was detected, respectively, in the lumina of the smooth endoplasmic reticulum and in the Golgi apparatus. The effect of colchicine on the cytoplasmic translocation of fibrinogen could be due to a direct action of the drug on the membranes of the endoplasmic reticulum or could be the indirect result of the disruptive action of the drug on the microtubules.

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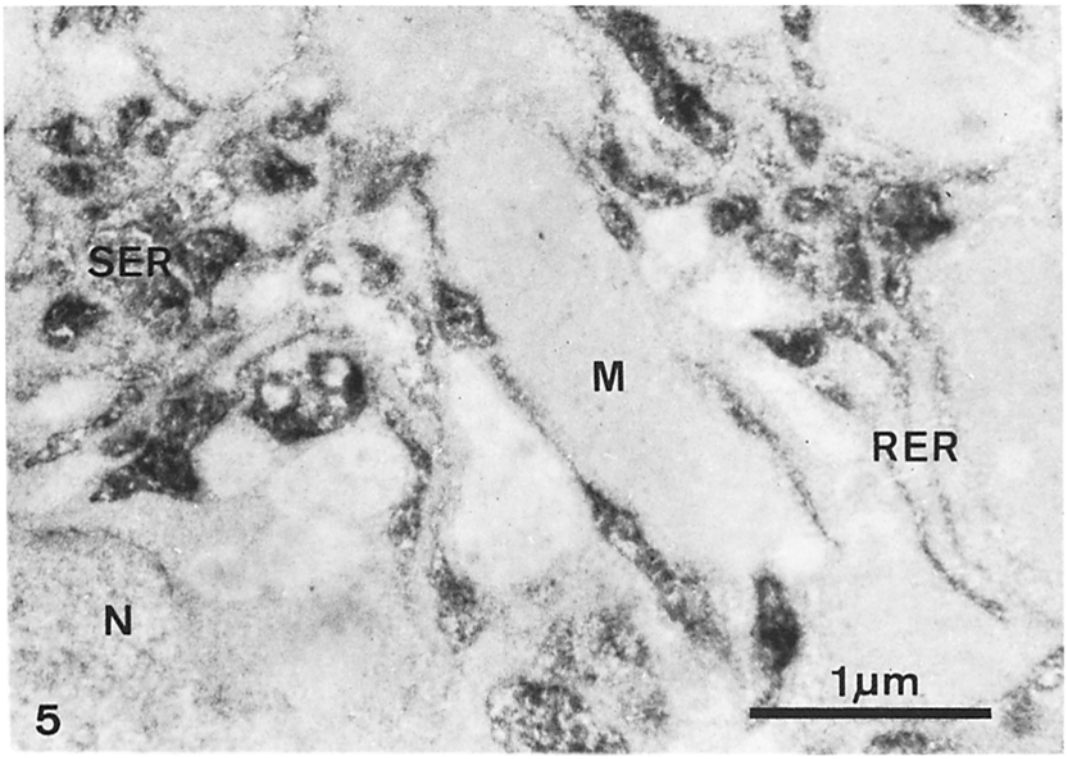
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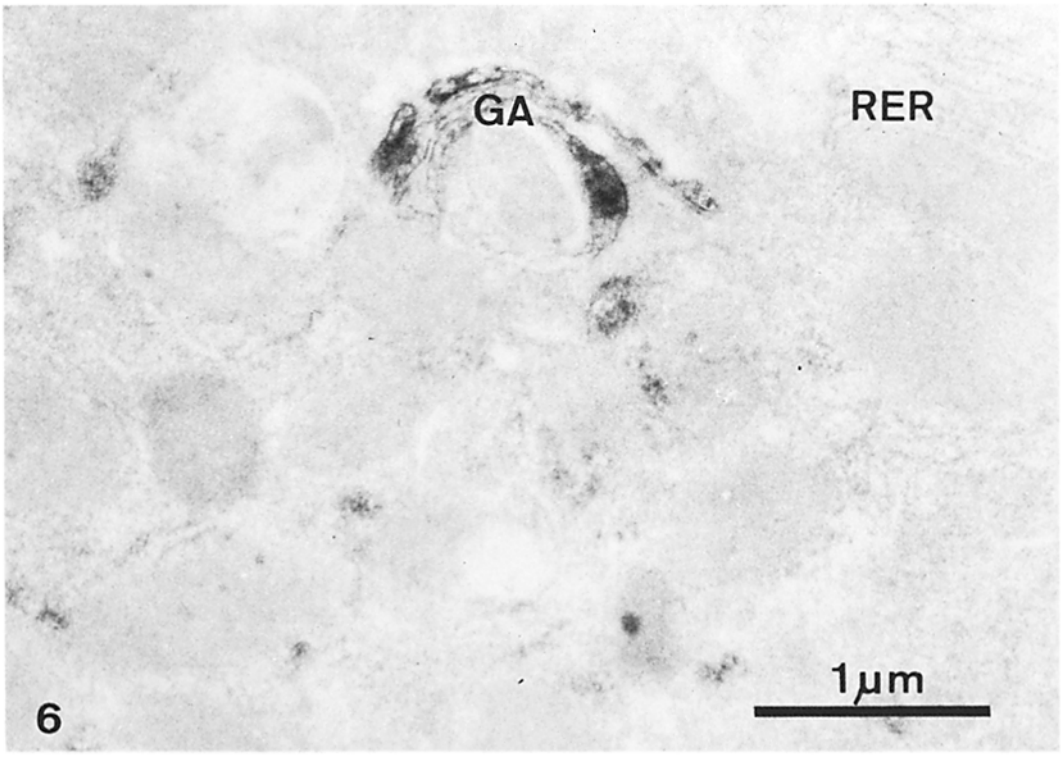
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FIGURE 5 Electron microscope appearance of a part of the smooth endoplasmic reticulum (*SER*) of a hepatocyte from a colchicine-treated rat 16 h after intraperitoneal injection of the drug. Fibrinogen is present in the lumina of the vesicles of *SER* and is detected in small amounts in the rough endoplasmic reticulum (*RER*). (*N*, nucleus; *M*, mitochondria.) × 32,000.

FIGURE 6 Electron microscope appearance of a Golgi apparatus (*GA*) observed in a hepatocyte from a colchicine-treated rat 24 h after intraperitoneal injection of the drug. Fibrinogen is detected in the saccules of the *GA*. No fibrinogen is detected in the rough endoplasmic reticulum (*RER*). × 31,000.