IN VIVO EFFECT OF COLCHICINE ON HEPATIC PROTEIN SYNTHESIS AND ON THE CONVERSION OF PROALBUMIN TO SERUM ALBUMIN

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ABSTRACT

Treatment of rats with 0.5–25 μmol/100 g body weight of colchicine for 1 h or more caused an inhibition of hepatic protein synthesis. This effect was not seen if the animals were exposed to colchicine for less than 1 h. The delayed inhibition of protein synthesis affected both secretory and nonsecretory proteins. Treatment with colchicine (15 μmol/100 g) for 1 h or more caused the RNA content of membrane-bound polysomes to fall but did not change the polysomal profile of this fraction. By contrast, the total RNA content in the free polysome cell fraction was increased, and this was due to the presence of more ribosomal monomers and dimers. Electron microscope examination of the livers from rats treated for 3 h with colchicine showed an accumulation of secretory vesicles within the hepatocytes and a general distension of the endoplasmic reticulum. Administration of radioactive L-leucine to the rats led to an incorporation of radioactivity into two forms of intracellular albumin which were precipitable with antiserum to rat serum albumin but which were separable by diethylaminoethyl-cellulose chromatography. One form has arginine at the amino-terminal position and is proalbumin, and the other form, which more closely resembles serum albumin chromatographically, has glutamic acid at its amino terminus. Only proalbumin was found in rough and smooth endoplasmic reticulum fractions and in a Golgi cell fraction which corresponds morphologically to mostly empty and partially filled secretory vesicles. However, in other Golgi cell fractions which were filled with secretory products, both radioactive proalbumin and serum albumin were found. This indicates that proalbumin is converted to serum albumin in these secretory vesicles just before exocytosis. Colchicine delayed the discharge of radioactive albumin from these filled secretory vesicles and caused an accumulation of both proalbumin and serum albumin within these cell fractions.

KEY WORDS albumin · proalbumin · secretion · Golgi · colchicine

The effects of colchicine on the physiology of the liver and on the secretion of plasma pro-

 J. CELL BIOLOGY © The Rockefeller University Press · 0021-9525/78/0501-0400$1.00

teins have been studied in detail in several laboratories. These studies have been performed in perfused liver, in liver slices, and in the intact animals. In the perfused liver, 10 μM colchicine does not affect a number of cellular parameters such as oxygen consumption, glucose production, intracellular adenosine triphosphate (ATP) levels, lipid synthesis, protein synthesis, or ureogenesis, but does inhibit the secretion of very low density lipoproteins (VLDL) and plasma proteins (15).

In rat liver slices, pulse-chased with radioactive L-leucine, colchicine (10^{-6}–10^{-5} M) inhibits plasma protein secretion, causes a concomitant retention of radioactive nonsecreted plasma protein within the slices, and does not affect protein synthesis for the first 50 min of incubation (22).

In the intact rat pretreated for 3 h with colchicine, VLDL release is inhibited, and when given twice (0.25 μmol/100 g body weight) at 0 min and 90 min for 150 min, colchicine does not affect protein synthesis, although higher doses (10-fold) have a slight inhibitory effect (27). In a further study, in which it was shown that colchicine inhibits lipoprotein secretion but does not interfere with the secretion of biliary phospholipids or of cholesterol, 1.25 μmol/100 g of this drug did not affect the incorporation of L-[^{3}H]leucine into the total proteins of the liver and serum combined (28). At higher concentrations of colchicine (25 μmol/100 g body weight) but for shorter time periods of exposure to the drug, colchicine inhibits secretion of plasma proteins, causes an accumulation of these nonsecreted proteins in Golgi-derived secretory vesicles, but does not impede the intracellular movement of the secretory proteins within the rough or smooth endoplasmic reticulum (1, 22).

There are conditions, however, under which colchicine affects both hepatic protein synthesis and secretion. For example, Dorling et al. (5) have reported that colchicine (0.625 μmol/100 g body weight) causes a decreased rate of appearance of radioactive albumin in the blood starting 60 min after administration of the drug and that this effect persists for 16 h. It was further shown by these investigators that 2 h after treatment with colchicine, there is an accumulation of L-[^{14}C]leucine-labeled albumin in the liver, but that there is no measurable increase in total intracellular albumin content, and it was concluded that the failure to accumulate any large amounts of proalbumin or of albumin in the liver is due to an inhibition by colchicine of the synthesis of proalbumin and its subsequent conversion to albumin.

This delayed inhibitory effect of colchicine on protein synthesis may be a consequence of a primary effect of this drug in impeding secretion, which may, in turn, activate feedback mechanisms aimed at slowing down the further synthesis of secretory proteins. On the other hand, it may be a more general effect caused by affecting the cell's metabolism. To distinguish between these and other possibilities, and to further elucidate the process by which colchicine inhibits hepatic secretion, we have studied in detail the effects of colchicine on the synthesis of hepatic proteins and on the secretion of rat serum albumin, with emphasis on the effect of this drug on the synthesis of serum albumin and on the intracellular processing of proalbumin to albumin.

MATERIALS AND METHODS

Animals

Male rats (90–190 g) were starved overnight before being used. In those experiments in which Golgi cell fractions were isolated, the rats were fed alcohol (0.6 g ethanol as a 50% solution/100 g body weight) by stomach tube 1 h before the administration of colchicine or radioactive L-leucine. Injections of colchicine or of radioactive amino acids were performed with the rats under light ether anesthesia and were administered via the femoral vein. Control rats received equal volumes of saline instead of colchicine.

Cell Fractionation Procedures

Rough and smooth endoplasmic reticulum cell fractions were prepared as described by Dallner and Ernster, (4) and Golgi cell fractions (22) and free and membrane-bound polysomes (23) were prepared by previously described procedures.

Isolation of Proteins

Radioactivity in serum proteins, albumin, and ferritin was determined as previously described (23, 24) using rabbit antiserum to rat serum proteins, to rat albumin, or to horse ferritin, which cross-reacts with rat ferritin. The cell fractions, usually from 5 to 8 g of liver, were treated with 0.5% sodium deoxycholate and centrifuged at 150,000g for 1 h before treatment with antibodies (22). When total hepatic albumin was isolated, the livers were homogenized in 5 vol of 0.01 M Tris-HCl, pH 7.4

in 0.154 M NaCl, treated with 0.5% sodium deoxycholate and centrifuged at 150,000*g* for 1 h. The albumins in the supernatant fraction were then obtained by immunoprecipitation with rabbit antiserum to rat serum albumin.

Proalbumin and hepatic albumin were obtained from the albumin immunoprecipitate by a modification of the method described by Judah and Nicholls (13). The immunoprecipitates, washed with saline, were treated with cold 5% trichloroacetic acid (TCA), placed in ice for 30 min, and then centrifuged. The TCA precipitates were washed three times with cold 5% TCA and extracted three times for 1 h each time at 4°C with 5 ml of 1% TCA in 95% ethanol. The pooled ethanolic extracts were dialyzed overnight at 4°C against 0.1 M Tris-HCl, pH 7.8. The dialyzed material was centrifuged to remove any insoluble protein and chromatographed on a 1 × 50 cm DEAE-cellulose column (Whatman DE 52) at room temperature. The sample was applied after mixing with 40 or 50 mg of rat serum albumin; the column was washed with 50 ml of 0.1 M Tris-HCl, pH 7.8; and the proteins were eluted with a 500-ml linear gradient of 0.1–0.3 M Tris-HCl, pH 7.8. Fractions of 3 ml were collected and assayed for radioactivity and for absorbancy at 280 nm.

Radioactive serum albumin was obtained from the plasma of a rat 60 min after injection with L-[^{3}H]leucine. The plasma was treated with 5% TCA and the precipitate was washed three times with cold 5% TCA and extracted with 1% TCA in 95% ethanol as described above. After dialysis, this material was chromatographed on DEAE-cellulose as described above.

Other Procedures

Amino-terminal analysis was performed by the manual Edman degradation method as modified by Iwanaga et al. (12). The intracellular albumins from six rats, treated with 15 μmol/100 g of colchicine, were labeled in vivo for 30 min with L-[G-^{3}H]glutamic acid (30Ci/mmol) and L-[U-^{14}C]arginine (0.324 Ci/mmol), as proalbumin is known to have *N*-terminal arginine (31, 20, 26,) and serum albumin contains *N*-terminal glutamic acid (3). Each rat received 0.1 mCi of L-[^{3}H]glutamic acid and 0.05 mCi of L-[^{14}C]arginine. Proalbumin and hepatic albumin were then isolated from combined Golgi and ER cell fractions by immunoprecipitation, alcohol extraction, and DEAE-cellulose chromatography as described above. Serum albumin, labeled with L-[^{3}H]glutamic acid and L-[^{14}C]arginine, was also prepared as described above but was obtained from the plasma of a single rat, 60 min after the administration of radioactive amino acids.

Nonradioactive albumin, used as a marker in DEAE-cellulose chromatography, was prepared from 50 ml of rat serum. The serum proteins were precipitated with 5% cold TCA, washed three times with cold 5% TCA, and extracted five times with 35 ml of 1% TCA in 95% ethanol. The pooled ethanolic extracts were dialyzed extensively at 5°C with 0.01 M Tris-HCl, pH 7.4 in 0.154 M NaCl. When this material was chromatographed at 5°C on Sephadex G100, it eluted as one major peak which, on immunoelectrophoresis against a multivalent rabbit antiserum to rat serum proteins, gave a single albumin precipitation band. The albumin thus obtained was dialyzed against water and stored at −20°C as a lyophilized powder.

Protein precipitates were dissolved in 1 ml of Soluene with 0.1 ml of glacial acetic acid. The samples were then counted with 10 ml of toluene scintillation phosphor in a Packard scintillation counter (Packard Instrument Co., Inc., Downers Grove, Ill.). Protein was assayed by the method of Lowry et al. (16) using bovine serum albumin as standard, and RNA was determined by the Orcinol method (17) using yeast RNA as standard.

Electron Microscopy

Liver samples were fixed in 2.5% formaldehyde, 2% glutaraldehyde in 0.1 M cacodylate buffer, pH 7.2 at 4°C for 1 h, and postfixed in 1% osmium tetroxide at 4°C for another hour. The specimens were dehydrated with acetone and embedded in Epon 812 mixture. Sections were stained with uranyl acetate and lead citrate. Electron micrographs were made with a Philips electron microscope, model 201.

Sources of Materials

Radioactive amino acids were purchased from Amersham-Searle Corp., Arlington Heights, Ill.; DEAE-cellulose (DE52) from Whatman, Inc., Clifton, N. J.; colchicine from Sigma Chemical Co., St. Louis, Mo.; and Soluene from Packard Instrument Co., Inc. Rat serum albumin was prepared by us from rat serum obtained from Pel-Freez Farms, Inc., Rogers, Ark.

RESULTS

General Effects of Colchicine on Hepatic Protein Synthesis

Rats were treated either with 25 μmol/100 g body weight of colchicine or with saline. At various time intervals, from immediately after injection to 4 h after the administration of the drug, L-[U-^{14}C]leucine was given intravenously for 10 min, the livers were removed, and the radioactivity incorporated into TCA-precipitable proteins and into total hepatic albumin was determined. As a measure of the uptake of radioactive L-leucine by the liver, the radioactivity in TCA-soluble fractions from the liver homogenate was also determined.

If L-[^{14}C]leucine was given immediately after or 15–30 min after colchicine, the drug was shown to

have no effect on the incorporation of radioactivity into TCA-precipitable protein or into hepatic albumin. After 1 h of colchicine pretreatment, however, there was a decrease in the levels of radioactivity in both TCA-precipitable proteins and in hepatic albumin, and this inhibition persisted for 4 h (Fig. 1). In some experiments, longer periods of colchicine treatment (6–8 h) were tested and resulted in similar effects. Keeping the animals for longer periods after colchicine pretreatment (25 μmol/100 g), such as overnight periods, frequently resulted in the death of the rats.

The L-leucine radioactivity in the TCA-soluble fractions, which represents mostly intracellular free L-leucine, increased after 1 h of colchicine pretreatment and reached a maximal level at about 3 h after colchicine treatment (Fig. 2, top). Dorling et al. have shown that the amino acid pools vary after colchicine treatment, with the intracellular specific radioactivity of L-leucine being raised 150% but that of glutamic acid, glycine, alanine, lysine, and histidine being decreased (5).

If L-[^{14}C]leucine was administered to rats pretreated for 3 h with colchicine, then a peak intracellular level of radioactive L-leucine was noticed within 2–3 min of L-[^{14}C]leucine administration. In saline-treated rats, the highest intracellular level of radioactive L-leucine was at 2 min after administration, and the "pulse" was sharper than in colchicine-treated rats, with lower intracellular levels of radioactive L-leucine at 3 min and at subsequent times up to 10 min after L-[^{14}C]leucine administration (Fig. 2, bottom).

To determine the in vivo doses of colchicine which cause this delayed inhibitory effect on protein synthesis, rats were pretreated with various doses of colchicine for 3 h and the rats were then pulse-labeled with L-[U-^{14}C]leucine for 10 min. At 0.5 μmol of colchicine/100 g body weight, the drug had a small inhibitory effect on the incorporation of radioactivity into both TCA-precipitable proteins and albumin. At concentrations of 5 μmol/100 g body weight or at higher doses up to 25 μmol/100 g body weight, colchicine progressively inhibited incorporation into these proteins (Fig. 3).

Effects of Colchicine on Free and Membrane-Attached Polysomes

Although albumin and other plasma proteins are synthesized on membrane-attached polysomes, it is not possible to generalize that only secretory proteins are made by membrane-at-

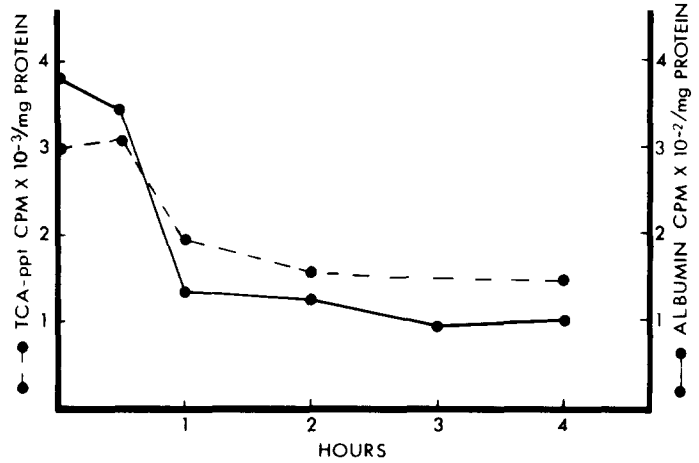


FIGURE 1 Incorporation of L-[U-^{14}C]leucine into TCA-precipitable protein and into albumin after various times of colchicine pretreatment. Rats were injected intravenously with 25 μmol/100 g body weight of colchicine. At the indicated times 9 μCi/100 g body weight of L-[U-^{14}C]leucine (324 mCi/mmol) was given in the femoral vein, and the livers were removed after 10 min, rinsed with 0.154 M NaCl, blotted, and homogenized in 0.154 M NaCl in 0.01 M Tris-HCl, pH 7.4. The incorporation of radioactivity into TCA-precipitable proteins and into albumin immunoprecipitate was determined as described in Materials and Methods. The dotted line represents the incorporation into TCA-precipitable protein and the solid line represents the incorporation into albumin.

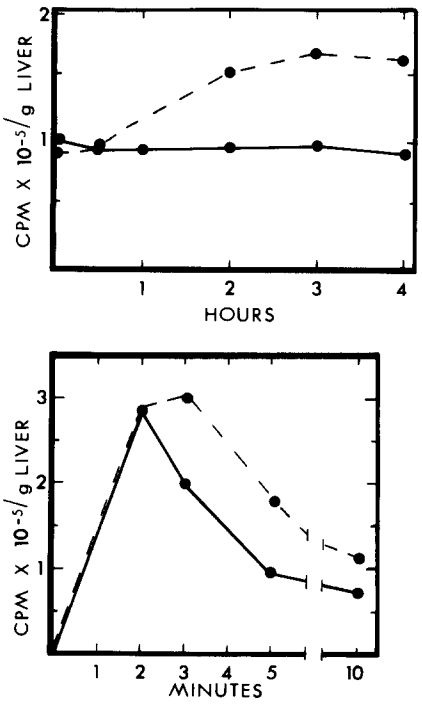


FIGURE 2 Intracellular hepatic levels of L-[U-^{14}C]leucine in control and colchicine-treated rats. *Top*: Rats were pretreated with 25 μmol/100 g body weight of colchicine and at the times indicated were pulse-labeled for 10 min with L-[U-^{14}C]leucine as in Fig. 1. The livers were removed, rinsed, and homogenized in 0.154 M NaCl, 0.01 M Tris-HCl, pH 7.4. The homogenate was treated with cold 10% TCA and the radioactivity in 10% TCA-soluble fraction was taken to be the intracellular level of L-[U-^{14}C]leucine. *Bottom*: Rats were treated with either 15 μmol/100 g body weight of colchicine or with saline 3 h before the intravenous injection of L-[U-^{14}C]leucine as described in Fig. 4. At the times indicated after the administration of radioactive L-leucine, the livers were removed and homogenized in 0.25 M sucrose. The radioactivity in a 10% TCA-soluble fraction from the homogenate was taken as the intracellular level of L-[U-^{14}C]leucine. The dotted lines represent the intracellular levels of L-[U-^{14}C]leucine in colchicine-treated rats and the solid lines represent that in saline-treated animals.

tached polysomes and that nonexportable proteins are made by free polysomes, inasmuch as it has been shown that some nonsecretory proteins are made on membrane-attached polysomes (21, 10). There is strong evidence, however, that secretory plasma proteins are finally assembled on membrane-attached polysomes (24, 30, 8, 9) before their vectorial discharge into the cisternae of the rough endoplasmic reticulum (ER) (25), and that some nonexportable proteins, such as ferritin, are preferentially synthesized, although not exclusively, by free polysomes (9, 24, 14). If the delayed inhibitory action of colchicine on protein synthesis is a feedback mechanism brought about by the delay in secreting plasma proteins, it may be expected that this drug will preferentially inhibit protein by membrane-attached rather than free polysomes. To determine whether this is true, rats were pretreated for various times from 0.5 to 3 h with colchicine, and the in vivo synthesis of proteins by both free and membrane-attached polysomes was measured 2–10 min after L-[U-^{14}C]-leucine administration. Colchicine had no effect on protein synthesis or on either free or membrane-attached polysomes after 0.5 h of colchicine pretreatment, but did inhibit both classes of polysomes after 1, 2, and 3 h of treatment. Colchicine inhibited the incorporation of radioactivity into TCA-precipitable proteins, in both free and membrane-attached polysomes at all times measured between 2 and 10 min after radioactive L-leucine administration. A representative experiment, taken at 3 h after colchicine treatment, when the effect is maximal is shown in Fig. 4.

After 3 h of treatment with 15 μmol/100 g body weight of colchicine, it was noticed that the recovery of RNA in the free polysomal cell fraction increased from 0.77 mg RNA/g liver to 1.15 mg/g liver, and that at the same time there was a decrease in the RNA content of membrane-attached polysomes from 1.31 to 0.54 mg RNA/g liver. However, as shown above, colchicine inhibited protein synthesis in both classes of polysomes. To analyze further this degradative effect of colchicine on the membrane-attached polysomes, rats were treated with the drug for various periods of time, choosing those times (0 and 30 min) at which colchicine does not affect protein synthesis and other times (1 and 3 h) when the delayed effect of colchicine on protein synthesis is maximal. Free and membrane-attached polysomes were isolated from the liver, and both the amounts of RNA/g liver and the profiles of the two classes of polysomes were determined. At zero time and at 29 min after colchicine, when the drug has little or no effect on protein synthesis, the RNA content of both free and membrane-attached polysomes was unchanged. Also unchanged was the profile of the polysomes measured as the percent of ribosomes which appear as either monomers,

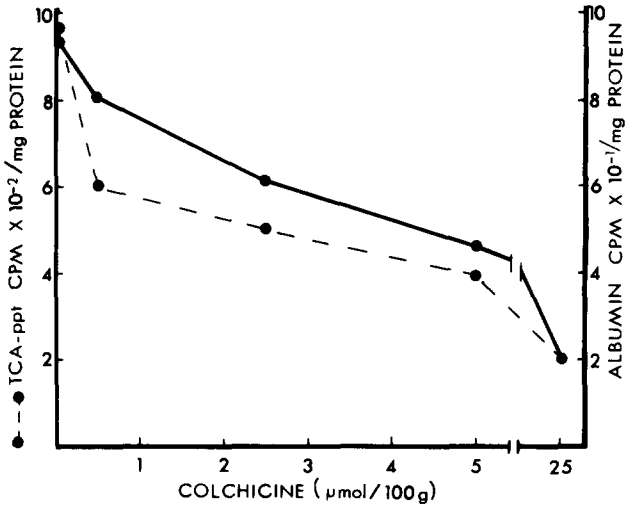


FIGURE 3 Effect of pretreatment with various concentrations of colchicine on the synthesis of albumin and TCA-precipitable proteins. Male rats (160–190 g) were administered various amounts of colchicine, and 3 h later 3 μCi/100 g body weight of L-[U-^{14}C]leucine (324 mCi/mmol) was given. After 10 min of the latter injection, the livers were removed and the incorporation of radioactivity into TCA-precipitable protein and albumin was determined as described in Fig. 1. The dotted line represents the incorporation into TCA-precipitable protein and the solid line that into albumin.

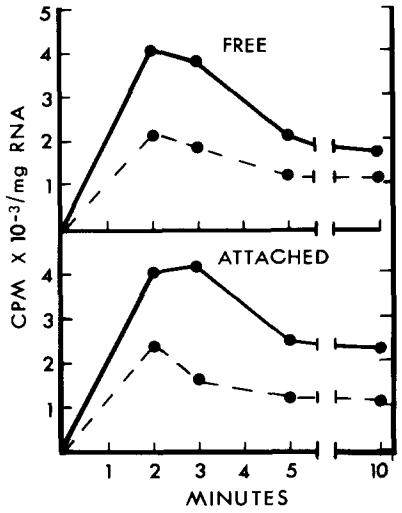


FIGURE 4 Time-course of incorporation of L-[U-^{14}C]leucine into nascent proteins of free and membrane-attached polysomes. Some rats (115–130 g) were pretreated with 15 μmol/100 g body weight of colchicine for 3 h while others received saline. L-[U-^{14}C]leucine (8 μCi/100 g, 324 mCi/mmol) was injected intravenously, and the livers were removed at 2, 3, 5, and 10 min after L-[^{14}C]leucine administration. After cell fractionation into free and membrane-attached polysomes, the radioactive incorporation into TCA-precipitable proteins was determined. The solid lines indicate the control and the dotted lines indicate the colchicine-treated rats.

dimers, or larger than dimers.

However, after 1 and 3 h of colchicine treatment, the amount of RNA/g liver in the free polysomes went up from 0.77 mg/g liver at zero time to 1.11 mg/g liver at 1 h and 1.15 mg/g liver at 3 h. At the same time, the profile of these polysomes exhibited a large increase in the number of monomeric and dimeric ribosomes (Table I). By contrast, the membrane-attached polysomes showed a decrease in RNA content at 1 and 3 h after colchicine treatment (1.31 mg/g to 0.6 and 0.54 mg/g) but showed no change in polysomal profile as evidenced by the fact that the percent of monomeric or dimeric ribosomes remained relatively unchanged (Table I).

It appears that degranulation of the rough ER occurs after lengthy colchicine treatment, and that some of the ribosomes which are released from the rough ER may appear as monomers or dimers together with the free polysomal cell fraction. Because more RNA is lost from the membrane-attached polysomes than is recovered in the free polysomal fractions (Table I), it is possible that the other ribosomes released from the membranes may remain undetected in the cytoplasm as ribosomal subunits, or may become degraded.

TABLE I

Polysomal Profile of Free and Membrane-Attached Polysomes of Colchicine-Treated Rats

Time after colchicine	Free				Membrane-attached			
	RNA	Monomers	Dimers	>2	RNA	Monomers	Dimers	>2
(min)	*(mg/g)*		*(%)*		*(mg/g)*		*(%)*	
0	0.77	5.6	8.9	85.5	1.31	9.8	12.8	77.4
29	0.79	4.6	6.1	89.3	1.11	11.0	10.4	78.6
60	1.11	9.1	18.0	72.9	0.60	8.2	7.5	84.3
180	1.15	9.7	18.0	72.3	0.54	11.4	11.0	77.0

Rats (180–190 g) were injected with 15 μmol/100 g body weight of colchicine and at the times indicated the livers were removed and fractionated into free and membrane-attached polysomes. The polysomes were resuspended in water, and aliquots were taken for RNA analysis. Polysomes corresponding to ~50 OD_{260} units were then layered on a 10–40% sucrose gradient containing 0.01 M Tris-HCl, pH 7.4, mM $MgCl_2$, and 25 mM KCl, and centrifuged for 105 min at 25,000 rpm in a Beckman SW27 rotor. The samples were collected by flotation (3 ml/min), and the optical density profile at 260 nm was recorded on a chart (3 min/ml). The polysomal profiles thus obtained were traced on 100% rag paper, and the areas on the tracings which corresponded to ribosomal monomers, dimers, and forms larger than dimers were cut out and weighed. The data presented are given as the percent of the total polysomal profile which appears as either ribosomal monomers, dimers, or as aggregates larger than dimers. The amount of polysomal RNA recovered per gram liver is also shown and is the average value obtained from two separate experiments with two animals at each time-point. The polysomal profile was only taken in the second experiment, and the values given are the averages from two separate rats.

Effect on Synthesis of Specific Hepatic Proteins

To determine whether pretreatment with colchicine affects the synthesis of all hepatic protein or more specifically inhibits only the synthesis of secretory proteins, rats were treated with colchicine for 3 h and the incorporation of L-[U-^{14}C]-leucine into different hepatic protein fractions was examined. Inhibition of radioactive incorporation by colchicine was seen in the total TCA-precipitable proteins obtained from the liver homogenate and in protein obtained from the deoxycholate-soluble endoplasmic reticulum, which represents a mixture of ER membrane proteins and any other proteins, mostly secretory ones, which are sequestered within the cisternae of the ER (Table II). An equal level of inhibition by colchicine was also seen both in the nascent plasma proteins contained within the ER and in the proteins residing in the soluble cell-fraction. The soluble cell fraction contains not only soluble nonsecretory protein, but may also contain secretory proteins which may have been released into this fraction during homogenization due to the rupture of ER and Golgi vesicles. To eliminate the secretory proteins from this fraction, the soluble cell fraction was treated with a multivalent rabbit antiserum to rat plasma, and the resulting supernate, containing mostly soluble nonsecretory proteins, was assayed for radioactivity. The results demonstrate that the synthesis of these soluble nonsecretory proteins was also inhibited by pretreatment with colchicine. Further evidence that colchicine inhibits the synthesis of nonsecretory proteins was obtained by showing that the synthesis of a specific nonsecretory protein, ferritin, was also depressed by colchicine (Table II). This experiment shows that pretreatment of rats with colchicine inhibits incorporation of radioactive L-leucine into most hepatic proteins and that the effect is not limited to secretory proteins. Taken together with the previous experiments which indicate that the delayed effect of colchicine on protein synthesis affects both free and membrane-attached polysomes, these results suggest that the effect of colchicine on protein synthesis is a general one which affects all or most hepatic proteins, and that it is not a specific feedback mechanism affecting only secretory proteins.

Conversion of Proalbumin to Hepatic Albumin

Within the hepatocyte, albumin exists in a precursor form, termed proalbumin, and is converted into serum albumin before secretion into the blood. Rat proalbumin has been characterized as containing a hexapeptide extension (Arg-Gly-Val-Phe-Arg-Arg) at the *N*-terminus of serum albumin (31, 20, 26). Recently, Ikehara et al. (11) have shown that proalbumin in vivo, is

TABLE II

Effect of 3 h of Colchicine Pretreatment on the Synthesis of Secretory and Nonsecretory Protein

	L-[U^{14}C]leucine incorporation in proteins		
	Control	Colchicine	% Inhibition
	(cpm/mg cell fraction protein)		
Homogenate	3,901	2,065	47
DOC-soluble ER	15,037	6,917	54
ER, plasma proteins	2,946	1,290	56
Soluble cell fraction	1,803	1,020	43
Soluble nonsecretory proteins	1,546	843	46
Ferritin	36	18	50

Rats (90–100 g) were pretreated for 3 h with 15 μmol/100 g body weight of colchicine and then 14.75 μCi/100 g of L-[U-^{14}C]leucine (324 mCi/mmol) was injected intravenously. After 20 min, the livers were removed and fractionated into various cell fractions. Radioactivity was determined in the TCA-precipitable proteins of the homogenate, endoplasmic reticulum (ER), and soluble fractions. Also determined were the radioactivities in nascent plasma proteins and in ferritin (see Materials and Methods). The radioactivity in soluble nonsecretory proteins was determined by treating the soluble cell fraction with antiserum to plasma proteins, to remove any secretory protein present in this fraction, and then by precipitating the remaining soluble fraction with 5% TCA. The incorporation of radioactivity into the various protein fractions is expressed as radioactivity per milligram of protein of the cell fraction from which the proteins were obtained.

Control rats received saline instead of colchicine. Each value is the average of four separate determinations using different rats for each determination.

converted into serum albumin while it is contained within Golgi-derived secretory vesicles, and studies by Edwards et al. show that in liver cell suspensions the conversion of proalbumin to serum albumin occurs in both the smooth ER and in the Golgi apparatus (6).

Because the conversion of proalbumin to albumin in vivo occurs in the Golgi cell fraction, and because this is the cellular site at which colchicine blocks secretion, we investigated in detail the effect of this drug on the processing of proalbumin to serum albumin, paying special attention to events which occur in the various Golgi fractions.

Rats were fed alcohol, as this procedure has been shown to increase the yield of the Golgi cell fraction and because the isolated Golgi fractions obtained by this method have been well characterized (7, 22). L-[^{3}H]leucine was then administered to saline as well as to colchicine-treated rats, and the livers were removed at various times, from 18 to 75 min, after L-[^{3}H]leucine administration. After cell fractionation into rough ER, smooth ER, and Golgi fractions 1, 2, and 3, nascent albumin was isolated from these cell fractions by immunoprecipitation with rabbit antiserum to rat albumin, and was separated into proalbumin and serum albumin by DEAE-cellulose chromatography. Also measured was the incorporation of radioactivity into albumin which had been secreted into the blood. This latter albumin fraction was also subjected to DEAE-cellulose chromatography.

In the rough and smooth ER cell fractions and in Golgi fraction 3, the only radioactive product in the antialbumin immunoprecipitate appeared to be proalbumin, as it elutes before serum albumin on DEAE-cellulose chromatography (13) and, as shown later, it contains *N*-terminal arginine instead of the *N*-terminal glutamic acid normally seen in serum albumin. There was little or no protein radioactivity which eluted together with rat serum albumin in these cell fractions (Fig. 5). By contrast, in Golgi fractions 2 and 1, there were two radioactive proteins obtained after DEAE-cellulose chromatography. The first radioactive protein, which is proalbumin, was eluted as a sharp peak before the elution of serum albumin. The second radioactive protein, which is more acidic, was eluted as a broader band, and its elution profile was close to but not identical to that of added rat serum albumin. The radioactive albumin obtained from plasma, in marked contrast to that from the various cell fractions, showed only one radioactive product which appeared in the more acidic position, and the radioactivity closely followed that of added rat serum albumin. There was no indication of a proalbumin radioactive peak in the plasma samples (Fig. 5).

Golgi fraction 3 has been previously shown to consist of flattened Golgi cisternae with distended rims which occasionally show some VLDL particles, and Golgi fractions 2 and 1 consist of rounded secretion vacuoles filled with VLDL particles and an amorphous dense matrix (22). These experiments, therefore, suggest that proalbumin exists throughout most of the secretory process but that, as the Golgi-derived secretory vesicles are filled (Golgi fractions 1 and 2), proalbumin is converted to another form of albumin.

Because the second radioactive peak obtained from Golgi fractions 1 and 2 does not elute

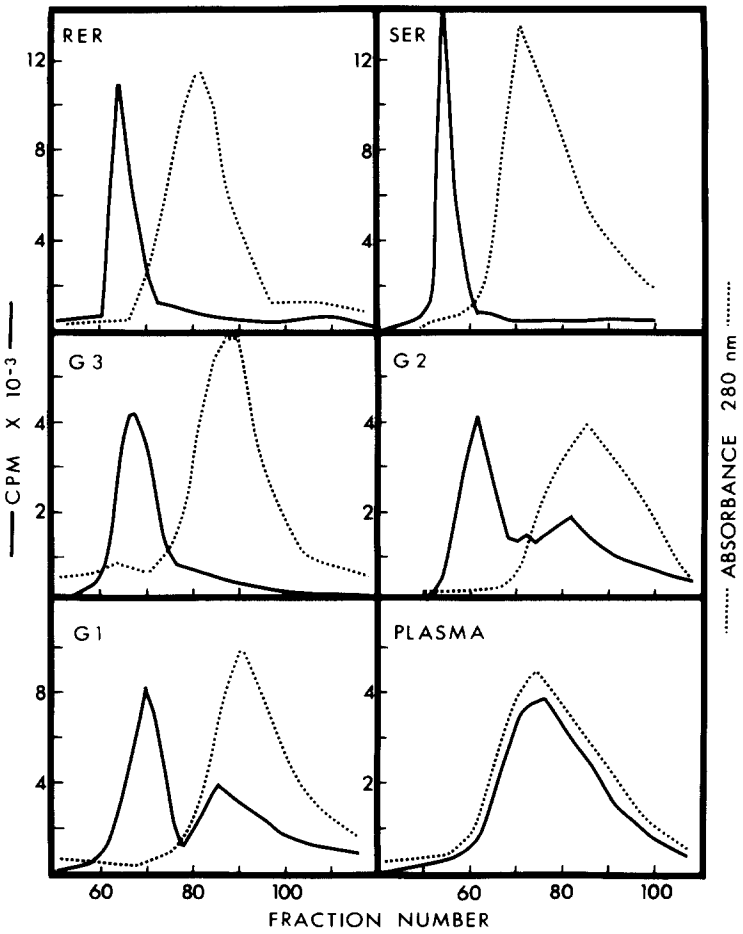


FIGURE 5 Radioactivity in proalbumin and albumin obtained from various liver cell fractions and from plasma. Rats were injected with 25 μmol/100 g body weight of colchicine. This was immediately followed by the administration of 250 μCi/100 g of L-[4,5-^{3}H]leucine (50 Ci/mmol). In the cases in which rough and smooth endoplasmic reticulum cell fractions were obtained, the livers were removed 20 min after L-[^{3}H]leucine injection. The rats from which Golgi fractions were obtained were fed alcohol (0.6 g/100 g body weight) 1 h before the administration of drugs. The livers were removed after 30 min. The plasma was obtained from rats which had been treated with radioactive L-leucine for 1 h. The detergent-treated cell fractions were immunoprecipitated with antialbumin, and the radioactive proteins thus obtained were then chromatographed on DEAE-cellulose together with 50 mg of added serum albumin, as described in Materials and Methods. The figures above only show some of the eluted fractions as the earlier fractions do not contain protein or radioactivity. Each fraction (3 ml) was assayed for radioactivity and for absorbance at 280 nm. Each figure represents a different but representative experiment using separate animals. The dotted lines show the absorbance at 280 nm of added serum albumin and the solid lines show the radioactivity. *RER*, rough endoplasmic reticulum; *SER*, smooth endoplasmic reticulum; and G1, G2, and G3 are three different Golgi fractions (described in the text).

identically with added rat serum albumin, and the radioactive albumin obtained from plasma does, this may indicate that the albumin obtained from these cell fractions may be chemically different from serum albumin. We therefore attempted to further identify the radioactive proteins obtained after DEAE-cellulose chromatography by determining *N*-terminal residues. It is known that proalbumin contains arginine in its *N*-terminus (31, 20, 26) and serum albumin contains glutamic acid (3). Because it is difficult to obtain sufficient amounts of these proteins from the various cell

fractions so as to identify the *N*-terminal amino acid by conventional analysis, L-[U-^{14}C]arginine and L-[G-^{3}H]glutamic acid were injected into colchicine-treated rats, and after 30 min the intracellular radioactive albumins were isolated from combined Golgi and ER cell fractions by immunoprecipitation and were separated into proalbumin and hepatic serum albumin by DEAE-cellulose chromatography. A manual Edman degradation *N*-terminal analysis was performed on the separated proteins (12), but was modified so that identification was performed by determining whether ^{14}C or ^{3}H was contained in the cleaved *N*-terminal amino acid residue. The first radioactive peak (proalbumin) obtained from DEAE-cellulose chromatography showed an *N*-terminal arginine, in that the radioactive amino acid obtained on the first Edman degradation contained 5,600 dpm of ^{14}C-radioactivity which was about 4% of the total ^{14}C-radioactivity in the proalbumin fraction. This same protein fraction contained 100,800 dpm of L-[^{3}H]glutamic acid radioactivity, but only 90 dpm of ^{3}H was released on the first Edman degradation step. The second peak, which eluted close to, but not identical with, serum albumin showed *N*-terminal glutamic acid, in that about 1% (258 dpm) of the ^{3}H-radioactivity was released by this procedure, but no significant [^{14}C]arginine was seen in the *N*-terminal position. An *N*-terminal analysis, using the above procedures on radioactive albumin obtained from the plasma, 60 min after the administration of L-[^{14}C]arginine and L-[^{3}H]glutamic acid, showed that the secreted albumin also contained only *N*-terminal glutamic acid. Thus, the second radioactive protein obtained from the liver appears to be similar to secreted albumin with respect to its *N*-terminal composition, and the reason for its slight chromatographic difference is not known.

These experiments indicate that the conversion of proalbumin to albumin occurs in filled Golgi-derived secretory vesicles (Fig. 5), which is the same cellular site at which colchicine causes an accumulation of nascent albumin (22). To determine whether colchicine affects the conversion of proalbumin to albumin, we followed, with time, the appearance of radioactive proalbumin and hepatic serum albumin in the 3 Golgi cell fractions and in the plasma obtained from control and colchicine-treated rats.

In Golgi fraction 3, which corresponds to mostly empty or only partially filled Golgi vesicles, radioactive proalbumin appears within 18 min of the administration of radioactive L-leucine. At this time and at 30 min, colchicine has little effect on the amount of radioactive proalbumin recovered from this cell fraction (Fig. 6). At later times, 45–75 min, there is a decline in the amount of radioactive proalbumin seen in this cell fraction, as presumably this protein passes to the other more filled secretory vesicles represented by Golgi fractions 2 and 1. At these later time intervals, 45 min and later, a small amount of residual hepatic serum albumin begins to appear in Golgi fraction 3, but it should be noted that at these time intervals there has already been substantial secretion of albumin out of the cell and into the plasma (Figs. 6 and 7).

In Golgi fractions 1 and 2, radioactive proalbumin also appears within 18 min and reaches a peak concentration at about 30 min. At later times, the amount of radioactive proalbumin in these cell fractions declines, and they are almost emptied within 60 min. At about the same time that proalbumin appears, radioactive hepatic serum albumin also is seen in these cell fractions, and this protein also appears to decline after 30 min and is mostly discharged by 1 h. It is difficult to see a precursor product relationship in these studies, but, clearly, as the radioactive proalbumin and hepatic albumin decline in Golgi fractions 1 and 2 (Fig. 6), the serum albumin secreted into the plasma increases (Fig. 7) and there appears to be no proalbumin in the plasma even at the earliest time interval measured.

Colchicine appears to have little effect in the first 18 min on the level of proalbumin or hepatic serum albumin in these Golgi cell fractions, although there was always slightly more radioactive protein in the Golgi cell fractions of colchicine-treated animals. Colchicine, however, appears to delay the discharge of proalbumin and hepatic serum albumin from these fractions. With increasing time, the radioactivity in both proalbumin and hepatic albumin is higher in the secretory vesicles of colchicine-treated rats because the levels of these radioactive proteins in the control animals has dropped with time as the albumin is secreted into the plasma, while the levels in the colchicine-treated rats have remained high. This is particularly noticeable in Golgi fraction 1 (Fig. 6). Colchicine does not specifically cause the accumulation of only one form of albumin.

Morphology

Treatment for 3 h with colchicine (25 μmol/100

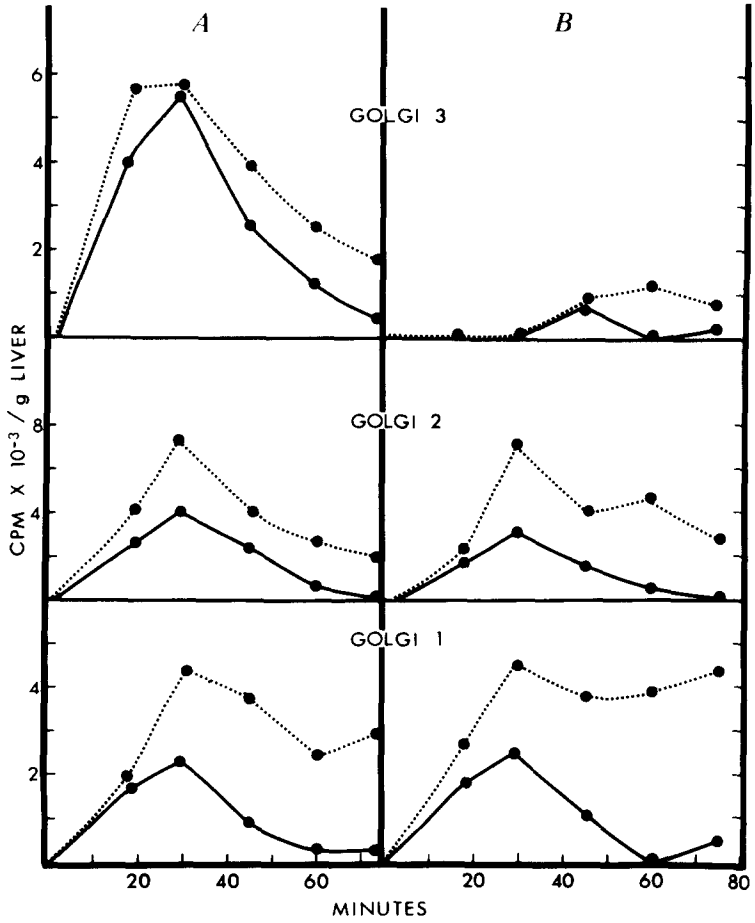


FIGURE 6 Time-course of appearance of nascent proalbumin and intracellular serum albumin in Golgi fractions of control and colchicine-treated rats. Rats were fed 0.6 g/100 g body weight of alcohol 1 h before the administration of colchicine and L-[4,5-^{3}H]leucine. Colchicine (or saline) was given intravenously, and this was immediately followed by the administration of 250 μCi/100 g of L-[4,5-^{3}H]leucine (50 Ci/mmol). The livers were removed at the indicated times after the injection of L-[^{3}H]leucine, and Golgi fractions were prepared. Proalbumin (column A) and intracellular albumin (column B) were separated by DEAE-cellulose chromatography as described in Fig. 5. The radioactivities in proalbumin and albumin were obtained after pooling the appropriate eluted fractions. The solid lines show the radioactivities in the control rats, and the dotted lines show those from colchicine-treated rats. Each point represents the value obtained from different animals. The 30- and 45-min time-points are the average of three experiments, and the others are from single experiments.

g body weight) inhibits the synthesis of secretory and nonsecretory proteins (Table II) and causes a decrease in the number of membrane-attached polysomes and an increase in the number of smaller ribosomal units (monomers and dimers) in free polysomes (Table I). Electron microscope examination of the colchicine-treated hepatocytes does not show a noticeable degranulation of the rough ER but does show a distension of the ER after this prolonged colchicine treatment. There is no other obvious or gross cytological damage to the cell, although a greater number of secretory vesicles are seen in the colchicine-treated animals (Figs. 8*A* and 8*B*).

DISCUSSION

Within a few minutes after intravenous administration to the rat, colchicine inhibits the secretion of albumin and other plasma proteins into the blood, and its effect is long lasting. Inhibition of

secretion is accompanied by a retention of the nonsecreted plasma proteins within Golgi-derived hepatic secretory vesicles, and previous kinetic studies show that colchicine affects the discharge, or exocytosis, of these secretory vesicles, but does not affect earlier secretory events such as intracellular movement of nascent secretory proteins from the rough to the smooth ER or from the smooth ER to the Golgi apparatus (22). Because the inhibitory effect of colchicine

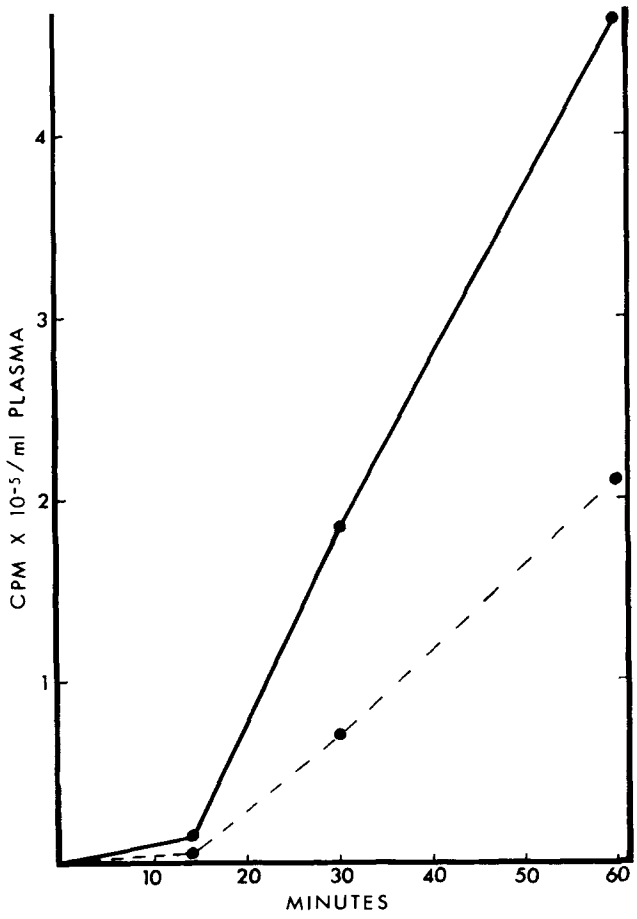
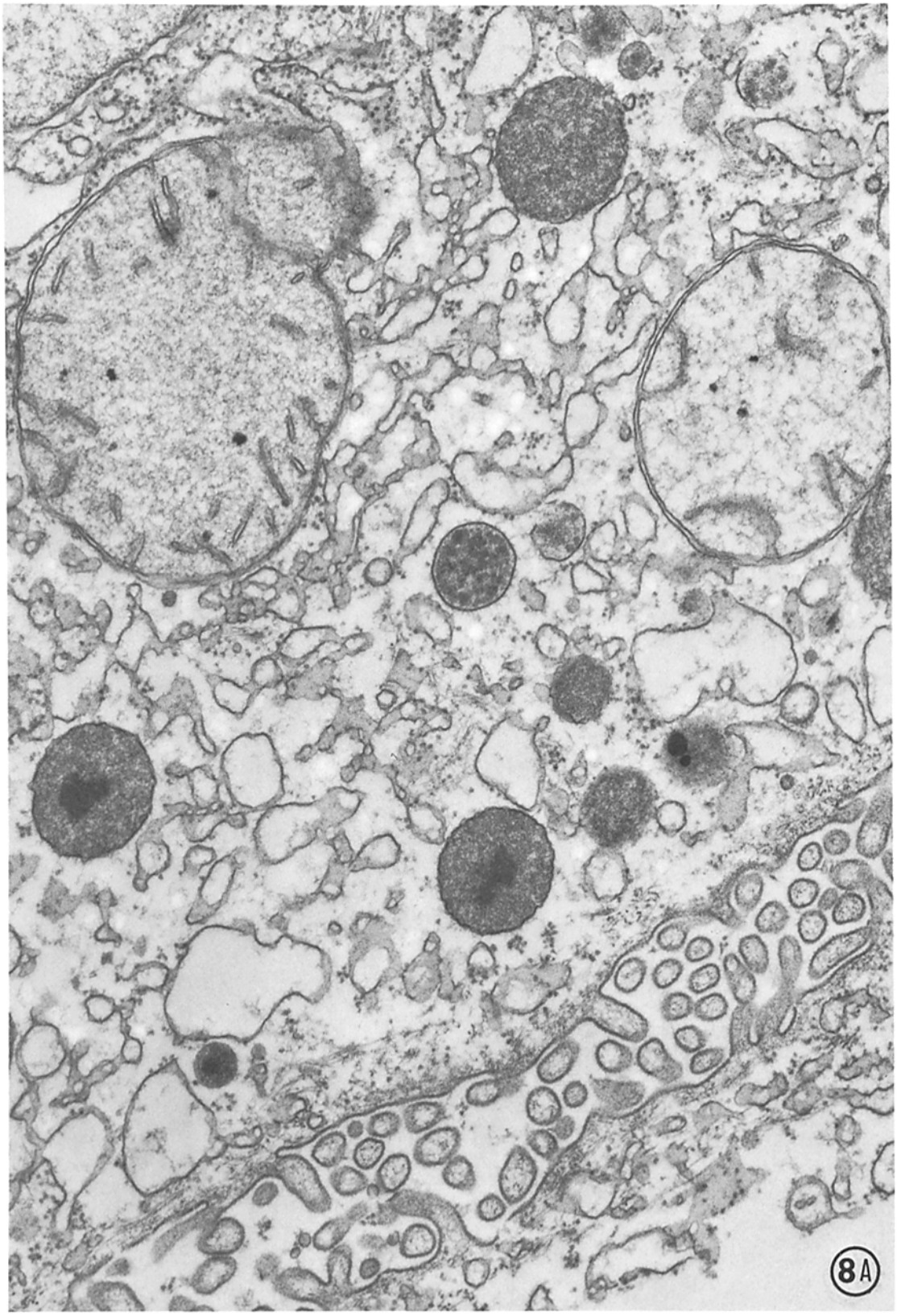
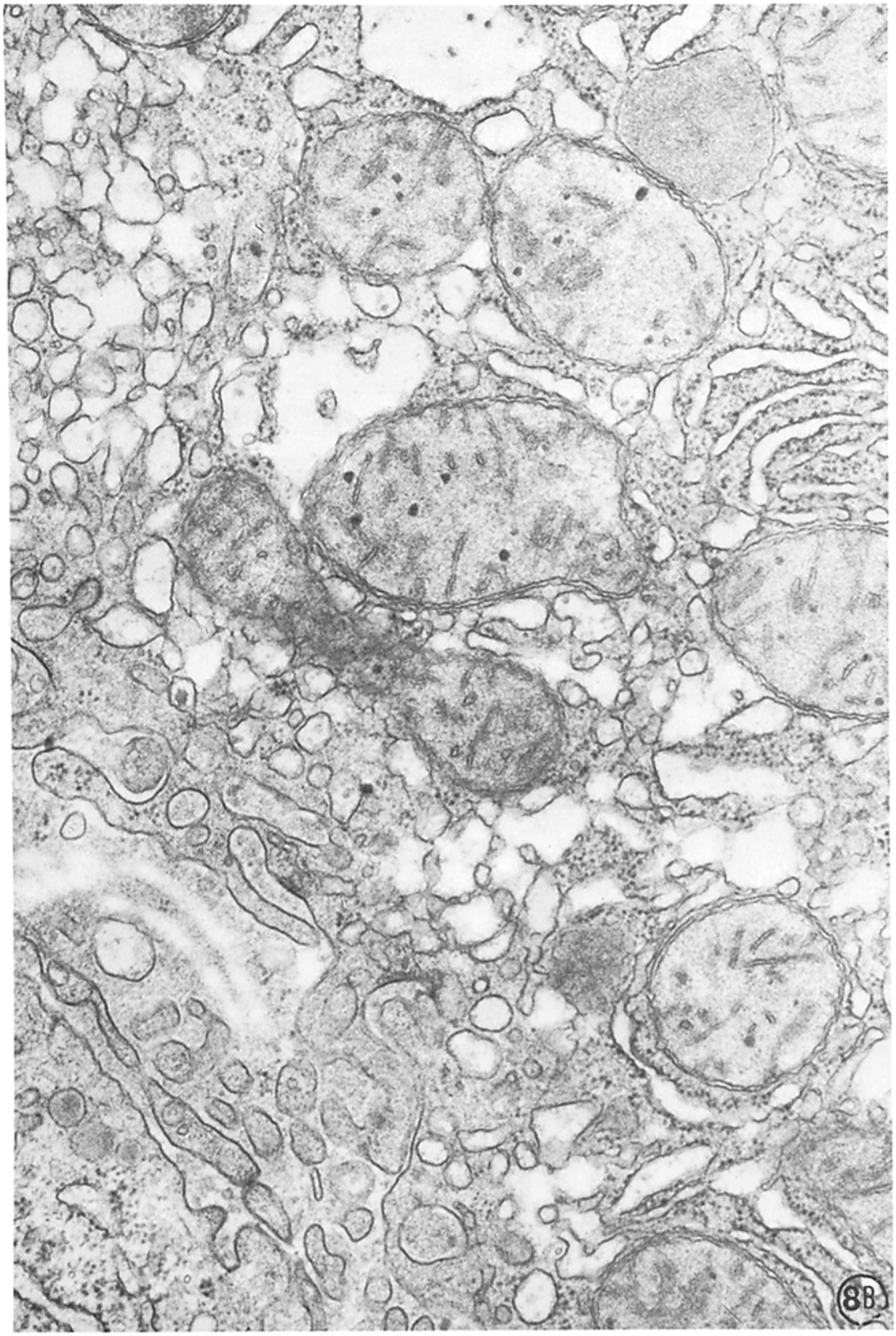


FIGURE 7 Time-course of incorporation of L-[4,5-^{3}H]leucine into plasma albumin as obtained by DEAE-cellulose chromatography. Saline- or colchicine-treated rats were injected with L-[4,5-^{3}H]leucine (as in Fig. 5), and at various time intervals blood was obtained from different rats by heart puncture. Radioactive albumin was obtained from the plasma by TCA-precipitation, alcohol extraction, and DEAE-cellulose chromatography as described in Materials and Methods. At no time was there any evidence of any radioactivity in proalbumin. The solid lines show the radioactivity in plasma albumin of control rats and the dotted lines show that from colchicine-treated rats.

FIGURE 8 Electron micrographs of normal and colchicine-treated rat hepatocytes. The rats were treated either with colchicine (25 μmol/100 g body weight) (Fig. 8*A*) or with saline (Fig. 8*B*), and the livers were removed after 3 h. All specimens were treated as described in Materials and Methods. Fig. 8*A* shows a large field in the cytoplasm of a hepatocyte treated with colchicine. The ER is distended and secretory vacuoles and peroxisomes are prominent. Fig. 8*B* shows a similar field from the saline-treated animals. × 45,000.


8A


8B

on secretion is prompt and can be localized at the level of exocytosis of secretory vesicles, and because one of the final processing steps in albumin synthesis and secretion in vivo, also occurs in this cell fraction (11), we have studied in detail the effect of colchicine on the conversion of proalbumin to albumin, to gain a better understanding of the final steps in albumin secretion and of the cellular mechanisms by which colchicine inhibits hepatic secretion.

Albumin, like some other secretory proteins (2), is probably first synthesized as a larger precursor protein with an octadecapeptide extension at the *N*-terminal end of proalbumin (29, 32), is vectorially discharged into the lumen of the rough ER (25) where it exists as proalbumin (Fig. 5), and is transported stepwise to the smooth ER and to the Golgi apparatus (19). When the Golgi-derived secretory vesicles are filled with secretory material and are ready to be emptied from the cell, proalbumin is converted to albumin. It is interesting to note, however, that, although there is little or no conversion of proalbumin to albumin at earlier time-points (20–30 min) in the less filled vesicles (Golgi fraction 3), some conversion is noted at later time points. In the filled secretory vesicles represented by Golgi fractions 1 and 2, there is at all times a mixture of proalbumin and albumin (Figs. 5 and 6). What mechanisms activate the removal of the *N*-terminal hexapeptide from proalbumin in filled vesicles (Golgi fractions 1 and 2) but not in less filled Golgi vesicles (Golgi fraction 3) is not known. Also not clear is whether this cleavage is a one-step procedure removing all six *N*-terminal amino acids at once, or whether intermediate steps are involved. Our experiments measure only the known *N*-terminal amino acids of proalbumin and serum albumin and thus do not allow the measurement of intermediate steps. There does appear to be some slight chromatographic difference between nascent albumin found in the Golgi and serum albumin (Fig. 5), but these two albumins have the following similar characteristics. They both precipitate with rabbit antiserum specific to rat serum albumin, copurify with ethanolic extractions, coelectrophorese on sodium dodecyl sulfate (SDS)-urea polyacrylamide gels, and contain the same *N*-terminal amino acid, glutamic acid. This chromatographic difference observed may be due to the well-known ability of serum albumin to bind many substances in the blood. Recently, Peters and Reed (18), using different separation techniques, have also shown that, in addition to proalbumin, there may be another form of albumin in the hepatocyte, which, although structurally similar to serum albumin, behaves differently chromatographically. Thus, it is quite possible that another processing step in the conversion of precursor albumin to serum albumin may occur in the Golgi apparatus.

Although colchicine inhibits secretion of plasma proteins into the blood within a few minutes after administration (22), a distinct accumulation of nonsecreted albumin in the Golgi cell fractions is not noticed until after 20 min (Fig. 6) of administration, because colchicine does not impede the filling of these vesicles with nascent secretory proteins but only inhibits their discharge. This may explain why other workers, who have pulse-labeled with radioactive L-leucine for 20 min, after lengthy pretreatment of rats with colchicine (0.128 μmol/100 g body weight) have noticed inhibition of secretion into the blood but no accumulation of nascent albumin within the Golgi cell fractions (11).

That the effect of colchicine on albumin secretion described above occurs within minutes but the inhibitory effect of this drug on protein synthesis is delayed and is not noticed until 1 h after colchicine has been administered (Fig. 1) may suggest that the primary effect of colchicine in inhibiting secretion may influence other cellular mechanisms which may, in turn, lead to a delayed inhibition of protein synthesis. It may be further expected that this feedback inhibition of protein synthesis may be specifically aimed at slowing down the further synthesis of secretory plasma proteins which are prevented from being secreted by the action of colchicine. This, however, does not appear to be the case, as the delayed inhibition of protein synthesis by colchicine affects both membrane-attached polysomes, which are known to be active in the synthesis of secretory proteins (8, 9, 23, 30), and free polysomes (Fig. 4), which may be involved in the synthesis of other proteins. Other evidence is that the synthesis of soluble cytoplasmic proteins, which may as a class represent nonsecreted proteins, and also the synthesis of a specific nonsecreted protein ferritin, were inhibited by colchicine to about the same extent as was the synthesis of the secretory plasma proteins (Table II). Thus, if inhibition of secretion by colchicine does cause a feedback inhibition of protein synthesis, it does

not specifically affect the synthesis of plasma proteins, but is a more general mechanism also affecting other hepatic proteins. Another possibility is that the delayed inhibition of protein synthesis by colchicine is not tied to the earlier inhibition of secretion but is merely due to a toxic effect of this drug.

The initial effect of colchicine in inhibiting protein secretion is a widespread phenomenon which occurs in many cells, and although the mechanism of action is not yet well understood, it is usually ascribed to the antimicrotubular activity of this drug. Some possibilities to explain the accumulation of secretory vesicles in response to colchicine are that discharge of hepatic secretory vesicles may involve microtubules which may directly or indirectly act to move, or to place these vesicles in the correct juxtaposition necessary to achieve contact and fusion with the plasma membrane at the blood front; or that colchicine inhibits exocytosis by some other mechanisms which may or may not involve active participation of microtubules. The fact, however, that colchicine has effects on the hepatic cell other than inhibition of secretion, and that these effects may not be at first apparent because of delayed mechanisms, should lead to caution in interpreting results when animals or cells are treated with colchicine for long periods of time.

We thank Miss Vivian Sarantis for technical assistance in the morphological studies and Dr. A. Mazur for helpful comments during the preparation of the manuscript.

This paper was supported by National Institutes of Health grants AM17741 and HL09011.

Received for publication 14 September 1977, and in revised form 30 December 1977.

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