

EFFECTS OF COLCHICINE ON OSTEOBLAST IN RAT: AN ULTRASTRUCTURAL STUDY

BY

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ABSTRACT

The present study was an attempt to investigate the effects of colchicine on the ultrastructure of rat osteoblasts with special reference to the microtubular function *in vivo*. Rats were killed at intervals of 2, 4, 8, 12 and 24 hours after the colchicine injection (0.1 mg per 100 g of body weight, subcutaneously). At 2 and 4 hours after the injection, the secretory granules and small vesicles accumulated in the Golgi area of the osteoblasts. The dilated spherical cisterna of the rough endoplasmic reticulum and the large vacuoles appeared, being located in the periphery of the cell. Microtubules were rarely found in the cytoplasm. Small masses of microfilaments of 80 to 110 Å in diameter were found also in the cytoplasm. According to the special staining for collagen, it was indicated that the contents of the secretory granules might be collagen-like materials. At 8 hours after the injection, the shape of the osteoblasts transformed to a round profile and the autophagic vacuoles increased in the cytoplasm. At 12 and 24 hours after the injection, the osteoblasts seemed to be destroyed by the breakdown of the plasma membrane and by the increase of the autophagic vacuoles. It is suggested that colchicine affects the secretory process of the bone matrix and the cytoskeletal system of the osteoblasts by interfering with the structure and the function of the microtubules and colchicine also interferes with the function of the plasma membrane followed by the destruction of the osteoblasts.

INTRODUCTION

Colchicine is one of the microtubule disruptive agents with the activity of specifically binding tightly to the tubulin, the microtubule being composed of the protein dimer of the tubulin (Borisy and Taylor^{1,2}). Using this specific action of colchicine, the function of the microtubules in the various cells have been studied in detail by many investigators. The microtubules are associated with different functions, including the chromosome movement in cell division, intracellular transport of materials, development and maintenance of cell shape and cellular motility (Olmsted and Borisy,³ Wilson,^{4,5} Margulis⁶). The microtubules

were found in all eukaryotic cells by using various techniques such as electron microscopy and immunofluorescent microscopy (Brinkley *et al.*,⁷ Weber⁸). In the osteoblasts, Weinger and Holtrop⁹ reported on the existence of the microtubules.

In the matrix-forming cells of the hard tissue, Kudo¹⁰ and Ogura *et al.*¹¹ reported that by using autoradiography and electron microscopy colchicine inhibited the matrix secretion of the odontoblasts and the ameloblasts but did not affect the synthesis of the matrix. The same results were reported on the matrix secretion of the osteoblasts *in vitro* using the biochemical and morphological techniques (Diegelman and Peterkofsky,¹² Ehrlich and Bornstein¹³), Ehrlich

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*et al.*¹⁴⁾ and Scherft and Heershe¹⁵⁾). These studies suggest that the microtubules are important in the secretory process for the matrix formation in the hard tissue. On the other hand, Raisz *et al.*¹⁶⁾ showed that bone resorption stimulated by parathyroid hormone was blocked by colchicine in organ culture. The ultrastructural study of the osteoclasts in the organ culture showed that the osteoclasts decreased the ruffled border and so the activity of bone resorption seemed to be reduced by colchicine (Holtrop *et al.*¹⁷⁾). According to these *in vitro* studies, it is suggested that colchicine inhibits both the bone formation and resorption. However, it remains to be established whether colchicine shows the same inhibitory effects on the bone cells *in vivo*.

The purpose of the present study is to obtain basic information about the effect of colchicine on the rat osteoblasts *in vivo*. The experiments were performed to see the histological changes with light and electron microscope.

MATERIALS AND METHODS

Twenty-five male Wistar rats each weighing about 200 g were used. Injection of colchicine was performed subcutaneously with an aqueous solution (0.1%) of 0.1 mg of colchicine (Merck and Co., Ltd., West Germany) per 100 g of body weight. The control rats received an equal volume of physiological saline without colchicine. At

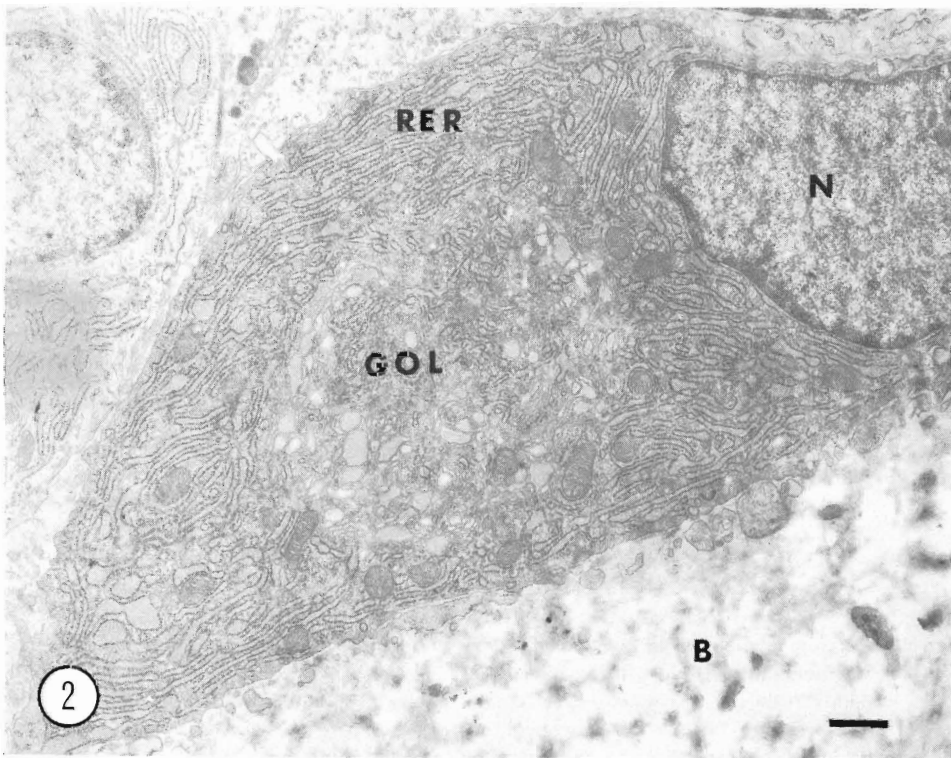
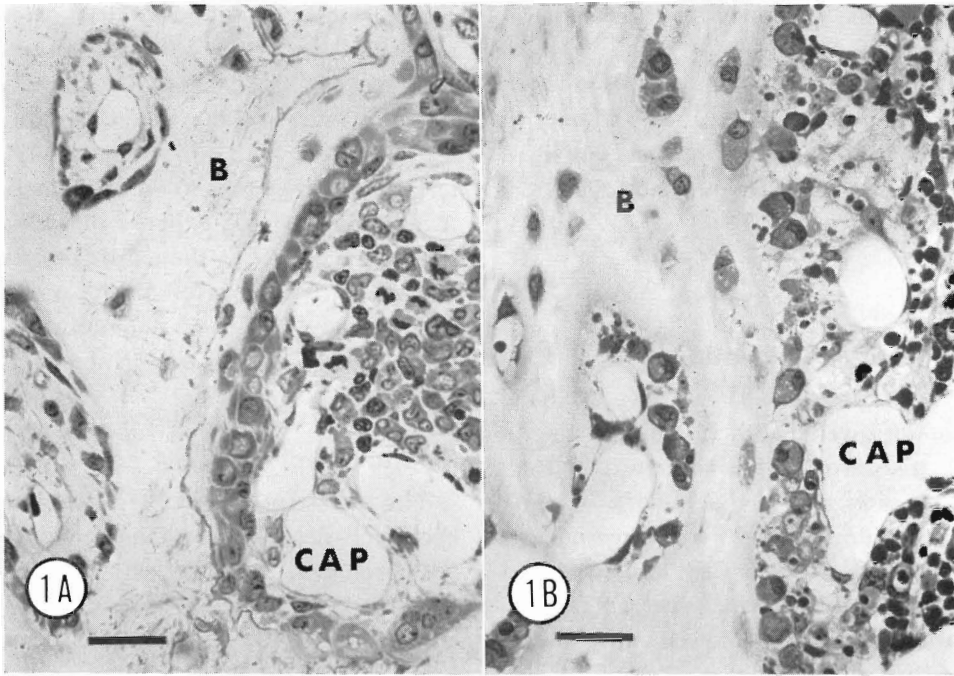
intervals of 2, 4, 8, 12 and 24 hours after the injection, the rats were anesthetized with an intraperitoneal injection of 0.5% pentobarbital sodium (Nembutal) and then fixed by perfusion of 2% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) containing 1.26% dextran, 1.05% glucose and 3 mM CaCl₂ at room temperature. The perfusion, made through the ascending aortae, lasted for about 30 minutes. The tibia and the humerus were then excised and decalcified for 3 weeks at 4° in 10% solution of EDTA in 0.1 M tris buffer containing 0.2 M sucrose (pH 7.4).

For light microscopy, the decalcified bone was embedded in paraffin and in glycol-methacrylate (GMA, Nissin EM Co., Ltd., Tokyo). The paraffin sections about 5 μ in thickness were stained by haematoxylin-eosin and Azan-Mallory staining. The GMA sections were cut with glass knives in a thickness of about 2 μ and stained with azure-eosin (Bennet *et al.*¹⁸⁾).

For electron microscopy, the decalcified bone was post-fixed for 2 hours with 2% OsO₄ solution in 0.1 M s-collidine buffer. Then the materials were dehydrated in a series of graded acetone and embedded in Epon 812. Ultrathin sections, made with the Porter-Blum MT-2 ultramicrotome (Du Pont Instrument, U.S.A.), were double-stained with uranyl acetate and lead citrate. Some other sections were stained with 5% aqueous solution of phosphotungstic acid

Fig. 1. Light microscopic photograph of the osteoblasts taken from the proximal metaphysis of the humerus in rat. Azure-eosin-stained GMA section of 2 μ -thickness. (A) control and (B) 8 hours after the colchicine injection. In the control group, a layer of the osteoblasts covers the bone surface and the osteoblasts contain pale-stained area, presumably the Golgi area. At 8 hours after the injection, the shape of the osteoblasts changes to a round profile. Many granular materials are found among the osteoblasts and many vacuoles accumulate in the cytoplasm. B: bone matrix, CAP: capillary, Bar, 20 μ \times 500

Fig. 2. Electron micrograph of an osteoblast taken from the proximal metaphysis of the tibia in the control rat. The abundance of rough endoplasmic reticulum (RER) and a well developed Golgi apparatus (GOL) show the characteristics of the osteoblasts. N: nucleus, B: bone matrix, Bar, 1.0 μ \times 7300



for 30 minutes in order to detect collagen (Scherft and Heershe¹⁵). These sections were examined by the Hitachi HU-11A or HU-12 electron microscope. The bone examined in the present study was obtained from the proximal metaphysis of the tibia and humerus.

RESULT

Light microscopic observations

In the control group (Fig. 1A), the bone surface was covered with a layer of the osteoblasts. This layer was usually one-cell deep. In other sites where the bone was being rapidly laid down, there were more than one layer in which the cells were closely packed and came contact with each other. The cytoplasm of osteoblasts showed pronounced basophilia and contained a pale-stained area near the nucleus, which was considered as the Golgi area.

Eight hours after the colchicine injection (Fig. 1B), significant changes were observed in the layer of the osteoblasts. Many osteoblasts transformed their shape into a round profile and some of them moved from the bone surface to the medullary cavity of the bone. Their close connection with the adjacent osteoblasts was dissociated and the osteoblasts were separated from each other. Many granular materials of varying sizes were found near the bone surface. The

round osteoblasts stained fairly pale compared to those in the control and the Golgi area seemed to be enlarged. Many small vacuoles and fibrillar materials were observed in the cytoplasm. These fibrillar materials stained similar to the bone matrix by Azan-Mallory staining, hence they might be the substance having the nature of collagen. Consequently, the layer of the osteoblasts was destroyed and their function seemed to be inhibited after the colchicine injection.

Electron microscopic observations

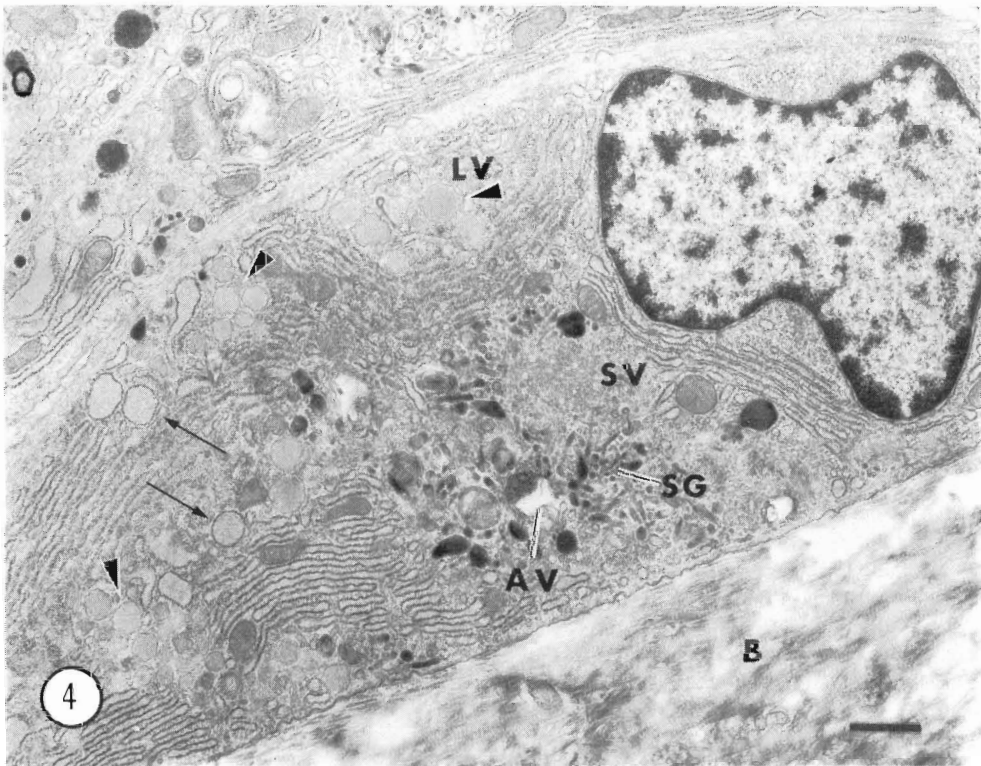
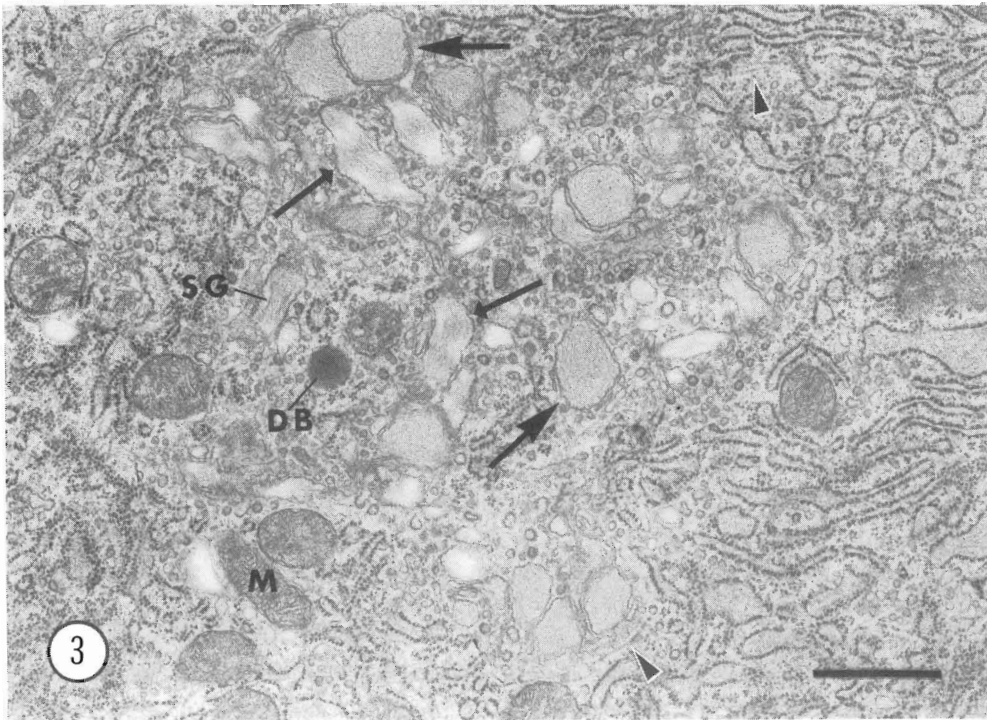
Control group

The ultrastructure of the osteoblasts has been well described by many investigators (Cameron,^{19,20} Weinstock,²¹ Leblond and Weinstock,²² Holtrop,²³ Scott and Glimcher,²⁴ Hancox²⁵). The bone surface covered with a layer of closely arranged osteoblasts (Fig. 2), followed by layers of pre-osteoblasts and fibroblasts with numerous capillaries. On the surface of the osteoblasts adjacent to the bone matrix, many cytoplasmic processes extended into the bone to make contacts with the similar processes of the other bone cells. The rough endoplasmic reticulum (RER) was extensive and its cisterna was often in a parallel array while the others were irregularly dilated.

The juxta-nuclear area contained the Golgi apparatus which was a prominent component of the membranous structure of

Fig. 3. Details of the Golgi apparatus in the control osteoblast. Spherical vacuoles (large arrows) contain fine granular or fibrillar materials. Cylindrical vacuoles (small arrows) contain fibrillar materials roughly in a parallel array. Secretory granules (SG) contain also fibrillar materials but closely packed in a parallel array. A dense body (DB) appears around the Golgi apparatus. Arrow heads show the microtubules. Numerous small smooth-surfaced vesicles, probably derived as buds from the RER, are found. M: mitochondria, Bar, $1.0 \mu \times 16,000$

Fig. 4. Electron micrograph of the osteoblast taken from the proximal metaphysis of the tibia at 2 hours after the colchicine injection. The osteoblast seems not to be so affected but slightly dilated. Accumulation of small vesicles (SV) and secretory granules (SG) are noted. Large vacuoles (LV, arrow heads) and dilated spherical cisterna of the RER (arrows) are seen near the periphery of the cell. Autophagic vacuoles (AV) are also found in the cytoplasm. B: bone matrix, Bar, $1.0 \mu \times 9,000$



the osteoblasts (Fig. 3). The Golgi apparatus was composed of several stacks of flattened sacculus or cisterna arranged in a parallel array and of the Golgi-associated vacuoles. The large spherical vacuoles (Fig. 3, large arrows) appeared near the distended ends of the sacculus and contained fine granular or fibrillar materials. There were also cylindrical vacuoles in the same area containing fibrillar materials in a roughly parallel array (Fig. 3, small arrows). These Golgi-associated vacuoles may accumulate and condense the secretory products synthesized in the RER, passing through from the "forming face" to the "maturing face" of the Golgi apparatus.

Among these large components of the Golgi apparatus, many small smooth-surfaced vesicles about 400 to 800 Å in diameter were observed. They may be the "transitional vesicles" (Jamieson and Palade²⁶) which transport the secretory products from the RER to the Golgi apparatus.

Elongated granules of various size were found near the Golgi apparatus. They contained fibrillar materials more closely packed in a parallel array than that of the cylindrical vacuoles. These granules may be the secretory granules containing procollagen, a precursor of collagen, as revealed by Weinstock and Leblond.²⁷

Microtubules were found in all osteoblasts in the control group examined in the present study. Microfilaments were also found and scattered throughout the cytoplasm. Bundles of parallel microfilaments were seen along the cell membrane and in the cytoplasmic process.

Colchicine-administrated groups

1. *Two hours after the injection*

At two hours after the colchicine injection, the ultrastructural changes appeared in the osteoblasts (Fig. 4). The shape of the osteoblasts were not so changed, but it tended to be dilated. The intercellular space between the adjacent osteoblasts seemed to be enlarged slightly. The Golgi apparatus seemed to occupy a larger area in the cytoplasm than in the control and it extruded the RER to the periphery of the cell.

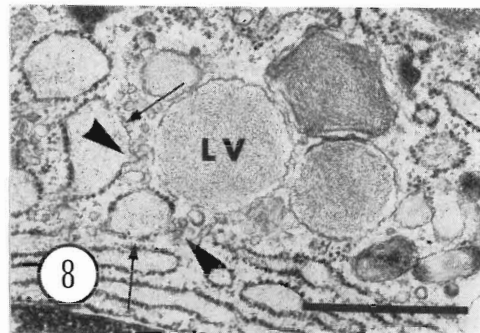
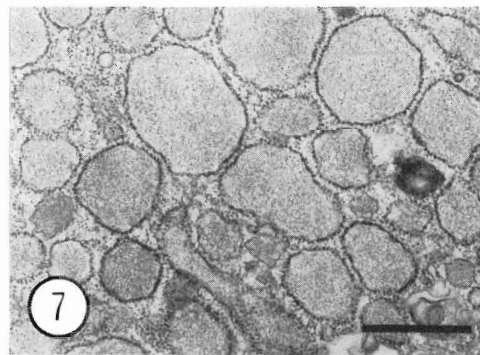
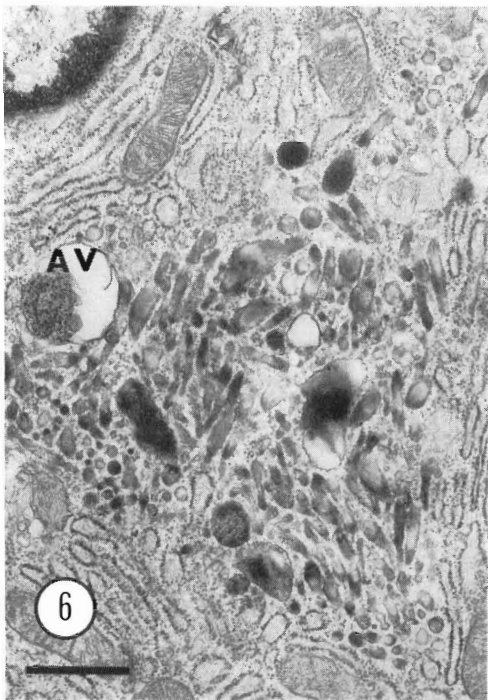
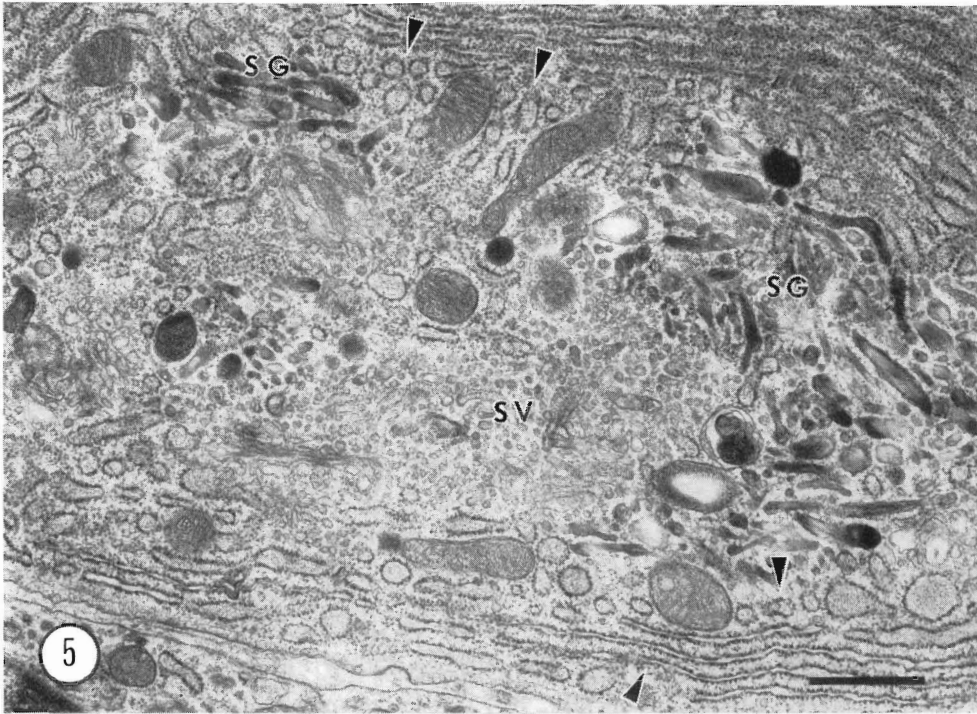
In the Golgi area, an accumulation of secretory granules containing electron-dense fibrillar materials were observed (Fig. 5). These materials did not show the periodicity like that of the collagen. These secretory granules were varied in size and appeared to be elongated, spindle-shaped, tubular, cylindrical, elliptical or spherical in profile (Fig. 6). They seemed to be distributed mainly in the Golgi area and not in the periphery of the cell (Fig. 4, 5 and 6).

Fig. 5. Details of the Golgi area of the osteoblasts at 2 hours after the colchicine injection. Accumulation of small vesicles (SV) and secretory granules (SG) is noted. Cisterna of the RER is lamellar and vesicular (arrow heads). Bar, $1.0 \mu \times 15,000$

Fig. 6. Electron micrograph of secretory granules at 2 hours after the colchicine injection. They are located in the Golgi area and show various profiles. No periodicity is found in the contents of secretory granules. An autophagic vacuole (AV) is also seen. Bar, $1.0 \mu \times 13,500$

Fig. 7. Electron micrograph of the dilated spherical cisterna of the RER at 2 hours after the colchicine injection. They vary in size and contain fine granular or fibrillar materials. Bar, $1.0 \mu \times 13,500$

Fig. 8. Electron micrograph of large vacuoles at 2 hours after the colchicine injection. Large vacuoles (LV) contain fine homogeneous granular materials. Many small vesicles are seen among the large vacuoles and they seem to derive from small protrusions (arrow heads) of the RER cisterna. These dilated spherical cisterna sometimes lack ribosomes (arrows). Bar, $1.0 \mu \times 22,000$



Many small vesicles also appeared and dominated a large part of the Golgi area (Fig. 5). These small vesicles were the "transitional vesicles" as shown in the control osteoblasts. The Golgi-associated vacuoles as shown in the control group were not found in the Golgi area.

The cisterna of the RER changed their shape into a tubular, lamellar or vesicular appearance as contrasted with the normal interconnecting canalicular structure in the control osteoblasts (Fig. 5). In the periphery of the cell distant from the Golgi apparatus, the cisterna of the RER dilated and became spherical (Fig. 4 and 5). These dilated spherical cisterna varied in size from 5,000 to 14,000 Å in diameter. They contained fine homogeneous granular or fibrillar materials with a medium electron-density.

Among these dilated spherical cisterna of the RER, large vacuoles were found (Fig. 4 and 8). They varied in size from 3,000 to 8,000 Å in diameter and contained fine homogeneous granular materials similar to that in the dilated spherical cisterna of the RER but the materials were slightly more electron-dense. They were surrounded by an irregular membrane and occasionally a translucent zone existed between the membrane and the materials. They might be derived from the RER, because the dilated spherical cisterna of the RER sometimes lacked ribosomes partially.

Many small vesicles often found in the surroundings of the large vacuole. These

small vesicles were similar to that found in the Golgi area and sometimes they were so close to the large vacuoles that they seemed to be taken up into the large vacuole (Fig. 8). In the RER near these vesicles, small protrusions of the cisterna and blunt endings, both free of ribosomes, could be observed. The small vesicles might bud off from the RER cisterna (Fig. 8, arrow heads).

In addition to these changes, several autophagic vacuoles appeared and were filled with various materials including the RER, mitochondria and secretory granules (Fig. 6). Dense bodies also appeared in the cytoplasm and some of them seemed to fuse with the autophagic vacuoles.

Microtubules could not be found in the cytoplasm of these osteoblasts, but in a rare case one or two intact microtubules were found in the cytoplasm.

Phosphotungstic acid (PTA) reaction appeared to bind selectively the contents of the secretory granules and the collagen fiber of the bone matrix (Fig. 9). However, the large vacuoles, small vesicles and cisterna of the RER were non-positive to PTA.

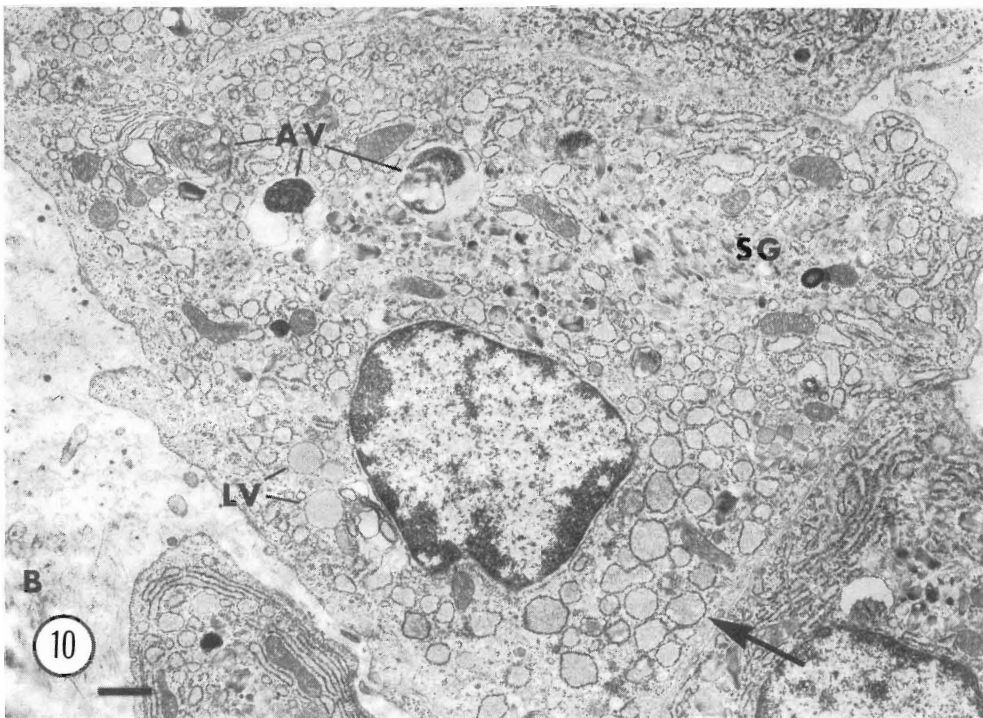
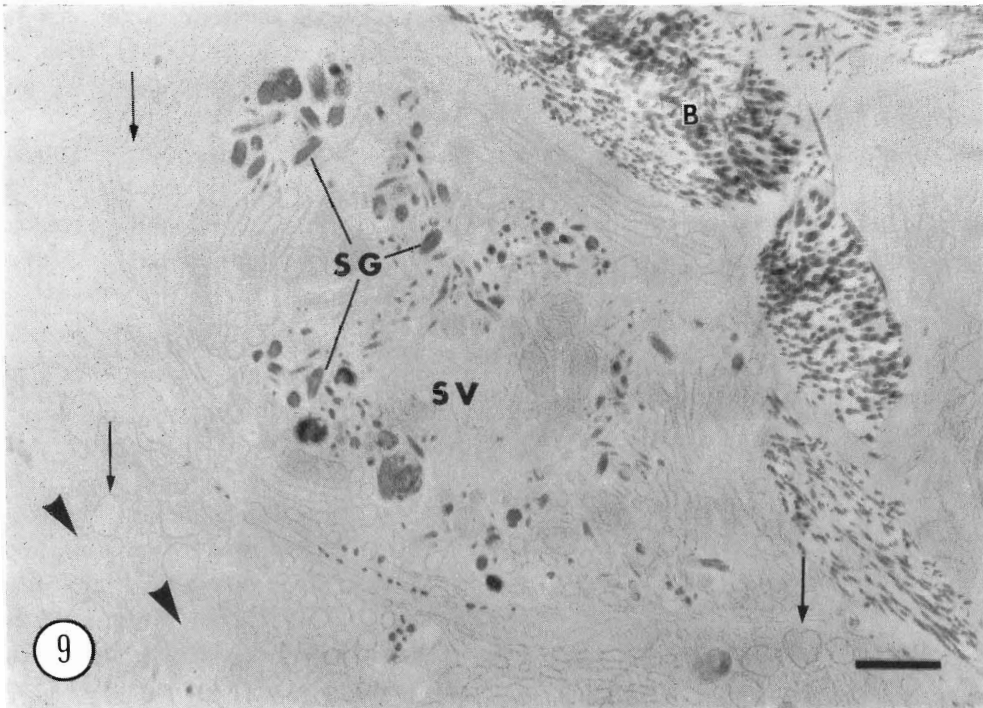
These findings indicate that the inhibition of the secretory process of the collagen in the osteoblasts occurred in the colchicine-administrated rat.

2. Four hours after the injection

The ultrastructural changes in the osteoblasts were similar to that 2 hours after the injection but the changes seemed to have

Fig. 9. Electron micrograph of the osteoblast stained with phosphotungstic acid (PTA) at 2 hours after the colchicine injection. Collagen of bone matrix (B) and secretory granules (SG) are positive to PTA but other membrane structures such as the spherical cisterna of the RER (arrows), large vacuoles (arrow heads) and small vesicles (SV) are non-positive. Bar, $1.0 \mu \times 11,000$

Fig. 10. Electron micrograph of the osteoblasts at 4 hours after the colchicine injection. Accumulation of the secretory granules (SG) as well as of the small vesicles are remarkable. The large vacuoles (LV), the dilated spherical cisterna of the RER (arrow) and the autophagic vacuoles (AV) increase in size and number. B: bone matrix. Bar, $1.0 \mu \times 6,800$



proceeded further (Fig. 10). The osteoblasts were dilated and the pattern of the osteoblasts moving from the bone surface into the bone marrow was more prominent.

Occasionally, microfilaments of from 80 to 110 Å in diameter were found in the cytoplasm (Fig. 11). They were randomly distributed and often aggregated in parallel bundles which varied in profile, long to short. Sometimes, several microfilaments seemed to connect with the mitochondria.

In several osteoblasts, some of their cytoplasmic organellae were lost (Fig. 12). The partial destruction of the plasma membrane appeared in this osteoblast. Occasionally, several microtubules were found and they run through the cytoplasm in random orientation (Fig. 12, arrows).

3. *Eight hours after the injection*

Osteoblasts transformed their shape into a round profile and the interconnection of the adjacent osteoblasts was lost and they were moved away from the bone surface (Fig. 13). Around these osteoblasts there were many cell debris and autophagic vacuoles.

In the cytoplasm, autophagic vacuoles containing various cytoplasmic organellae increased in number and size and they dominated a large part of the cytoplasm. Large vacuoles became enlarged and increased in number in the surroundings of the RER. The cisterna of the RER were dilated remarkably and accumulation of small vesicles and secretory granules in the Golgi area was also noted. Free ribosomes probably detached from the RER increased in number

and were seen scattered in the cytoplasm. Bundles of microfilaments of from 80 to 110 Å in diameter also appeared in some osteoblasts.

Some osteoblasts maintained their ultrastructure comparatively well (Fig. 14). They were elliptical or spindle-shaped cells when compared with the round cells as shown in Fig. 13.

4. *Twelve and twenty-four hours after the injection*

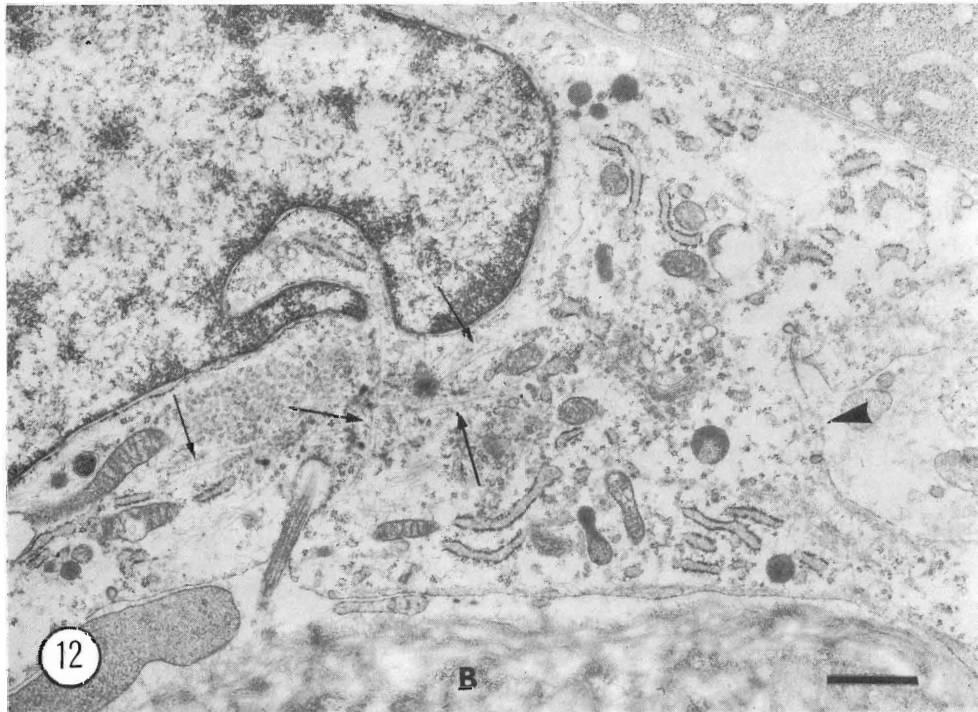
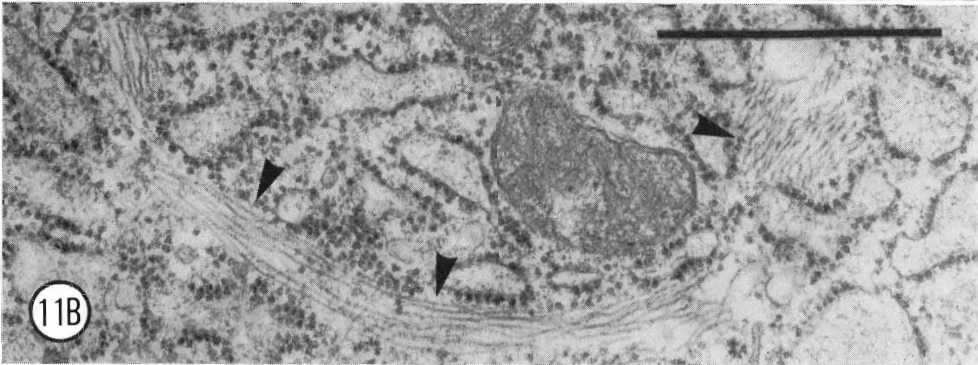
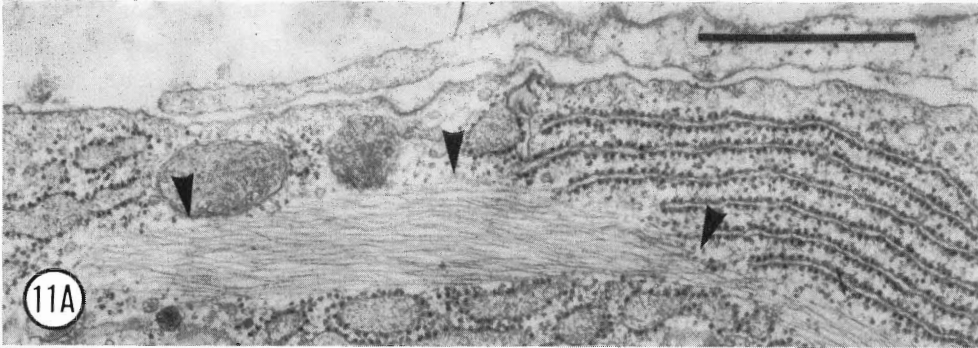
There were no remarkable differences in the osteoblasts between 12 and 24 hours after the colchicine injection. Degenerative changes proceeded markedly. Osteoblasts seemed to be destroyed by the breakdown of the plasma membrane and by the increase of the autophagic vacuoles. Many cell debris and autophagic vacuoles were seen scattered here and there in the bone marrow near the bone surface (Fig. 15). The destruction of the cytoplasmic process proceeded to the destruction of the osteoblast. The network of the cytoplasmic process between osteoblasts and the other bone cells might be also destroyed. Consequently, the osteoblasts disappeared from the bone surface and the bone marrow extracellular fluid seemed to come in contact directly with the bone surface.

Besides the cell debris and the autophagic vacuoles, many electron-dense fibrils appeared and were seen scattered in the bone marrow near the bone surface (Fig. 15, arrows). These fibrils aggregated in a profile of various types and sometimes showed a periodicity of about 200 Å (Fig. 15, inset).

Most of the bone surface were free from

Fig. 11. Higher magnification of the bundles of microfilaments of from 80 to 110Å in diameter (arrow heads) at 4 hours after the colchicine injection. Bar, 1.0 μ A, ×29,000 B, ×38,000

Fig. 12. Electron micrograph of the osteoblast at 4 hours after the colchicine injection. The osteoblast seems to lose their cytoplasmic organellae by the destruction of plasma membrane (arrow heads). Several microtubules (arrows) are present in the cytoplasm. B: bone matrix. Bar, 1.0 μ ×12,000



the layers of osteoblasts. However, few cells different from the osteoblasts were found in some areas of the bone surface: they were the several bone marrow cells, the cells undergoing mitosis or showing a metaphase arrest and the cells containing a small number of RER, small vesicles and occasionally microtubules (Fig. 16).

DISCUSSION

It has been reported that colchicine affects the secretory process in many tissues. Some examples were as follows: inhibition of the secretion of insulin (Malaisse *et al.*²⁸), thyroid hormone (Williams and Wolff²⁹), low density lipoprotein (Stein and Stein³⁰), lactose (Guerin and Loizzi³¹) and albumin (Redman *et al.*³²). Colchicine has been also shown to inhibit the secretion of collagen from the various cells such as osteoblasts (Diegelman and Peterkofsky,¹² Ehrlich and Bornstein,¹³ Ehrlich *et al.*,¹⁴ Scherft and Heershe¹⁵), odontoblasts (Kudo,¹⁰ Ogura *et al.*¹¹), tendon cells (Dehm and Prockop,³³ Olsen and Prockop³⁴), cartilage cells (Harwood *et al.*³⁵) and skin cells (Tranvska *et al.*³⁶).

Studies on the route of intracellular transport of collagen were conflicting. However, the classical pathway found in the various secretory cells (Palade³⁷) is now accepted as the major intracellular pathway of the collagen (Grant *et al.*³⁸) i.e. the polypeptide chains of collagen synthesized on the membrane-bound ribosomes are transferred into

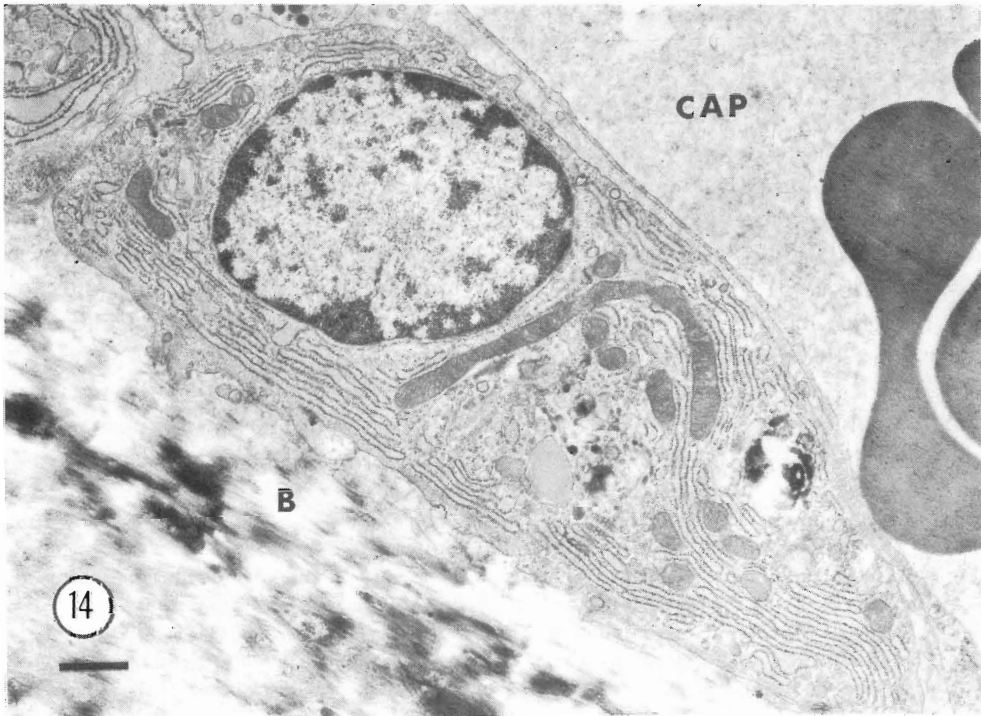
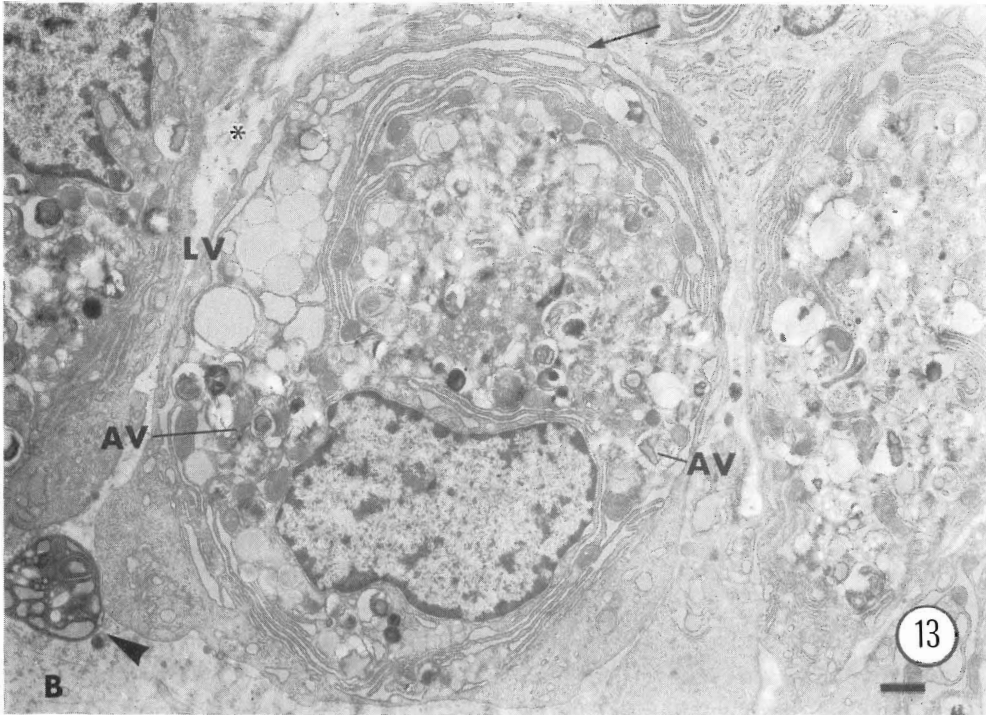
the cisterna of the RER and then transported by the smooth endoplasmic reticulum or small vesicles to the Golgi apparatus. In the Golgi apparatus, the collagen precursor is condensed and packed into the secretory granules and then transported to the plasma membrane and released from the cell.

The accumulation of the secretory granules after the colchicine injection did not appear in the periphery but in the Golgi area in the cell (Fig. 4, 6). This may indicate that the microtubules are involved in the pathway in which the secretory granules are transported to the plasma membrane. Previous studies on the collagen-secretion cells treated *in vitro* with colchicine support this speculation in the present study (Diegelman and Peterkofsky,¹² Dehm and Prockop,³³ Harwood *et al.*³⁵).

From the observation of the disappearance of the Golgi-associated vacuoles, it is assumed that the function of the Golgi apparatus was reduced with time after the colchicine injection. As the result of the reduction of the function of the Golgi apparatus, small vesicles that budded off from the RER could not associate with the sacculus of the Golgi apparatus and then they accumulated in the Golgi area (Fig. 5). Recently, Harwood *et al.*³⁵ reported that the Golgi fraction and the smooth microsomal fraction isolated from the tendon cells and cartilage cells increased in the presence of colchicine *in vitro*. These findings sup-

Fig. 13. Electron micrograph of the osteoblast at 8 hours after the injection. The osteoblasts transform their shape into a round profile and tend to away from the bone surface. Interconnections of adjacent osteoblasts are dissociated (asterisk). There are many cell debris and autophagic vacuoles (arrow head) around the osteoblast. Many autophagic vacuoles (AV) and large vacuoles (LV) are found in the cytoplasm. Cisterna of the RER is dilated markedly (arrow). B: bone matrix. Bar, $1.0 \mu \times 5,250$

Fig. 14. Electron micrograph of the osteoblast at 8 hours after the colchicine injection. This osteoblast is present at another site of the same section as Fig. 13 and does not show so remarkable a change compared with the osteoblast in Fig. 13. B: bone matrix. CAP: capillary. Bar, $1.0 \mu \times 8,800$



port the present observations that the accumulation of the secretory granules may cause the increase of the Golgi fraction and the accumulation of the small vesicles may cause the increase of the smooth microsomal fraction.

The appearance of the dilated spherical cisterna of the RER and the large vacuoles (Fig. 4, 7) may indicate that the synthesis of the secretory materials on the membrane-bound ribosomes is not inhibited by the colchicine administration. This is consistent with the previous findings about the colchicine-treated cells *in vitro* (Diegelman and Peterkofsky,¹² Dehm and Prockop³³).

PTA has been used for the detection of the carbohydrate in electron microscopy (Pease³⁹). Collagen is a glycoprotein containing galactose and glucose residues and has a great affinity to PTA (Weinstock and Leblond,²⁷ Scherft and Heershe¹⁵). When the sections were stained with PTA (Fig. 9), only the secretory granules and collagen of the bone matrix were positive and the other organellae such as the large vacuoles, cisterna of the RER and small vesicles were non-positive. Therefore, it is suggested that the contents of the secretory granules are collagen-like materials, probably procollagen.

The contents of the vesicles and the vacuoles other than the secretory granules could not be detected by this staining. However, Olsen and Prockop³⁴ recently reported that, by using the ferritin-labeled antibodies

specific for NH₂-terminal extensions of the procollagen, the ferritin-conjugate was located in the cisterna of the RER and the large Golgi vacuoles in the tendon cells. They also reported that the ferritin-conjugate was located in the "large smooth-surfaced vacuoles" which increased in number and size after the colchicine treatment. The morphological characteristics of these "large smooth-surfaced vacuoles" resemble those of the large vacuoles as shown in the present study. Accordingly, it is likely that the contents of the dilated spherical cisterna of the RER and the large vacuoles are immature secretory materials, probably procollagen that is not glycosylated.

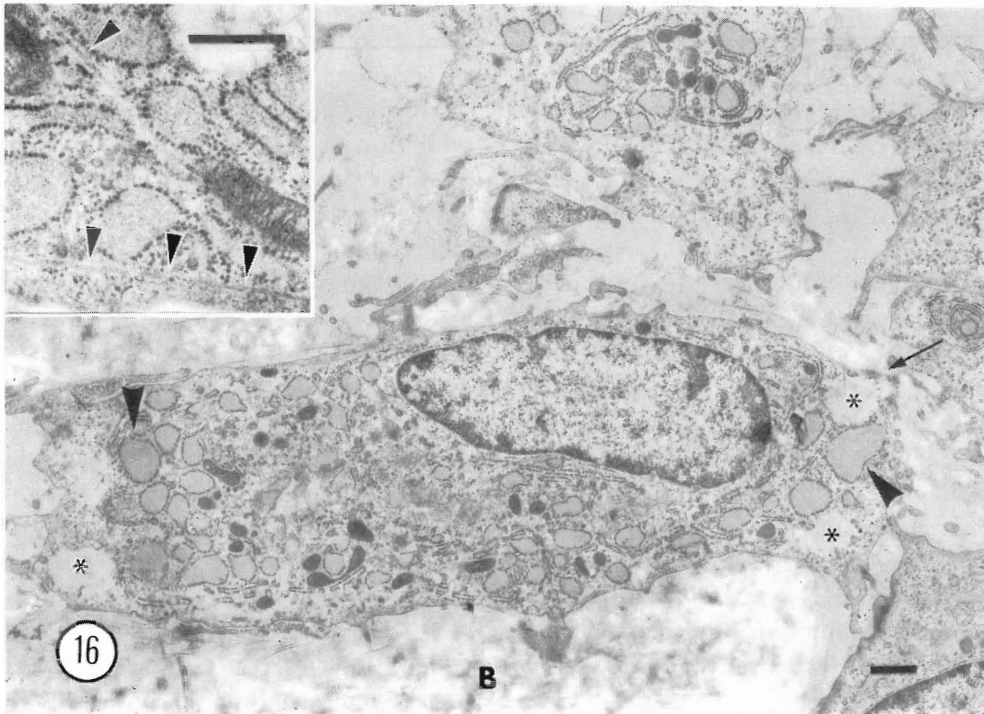
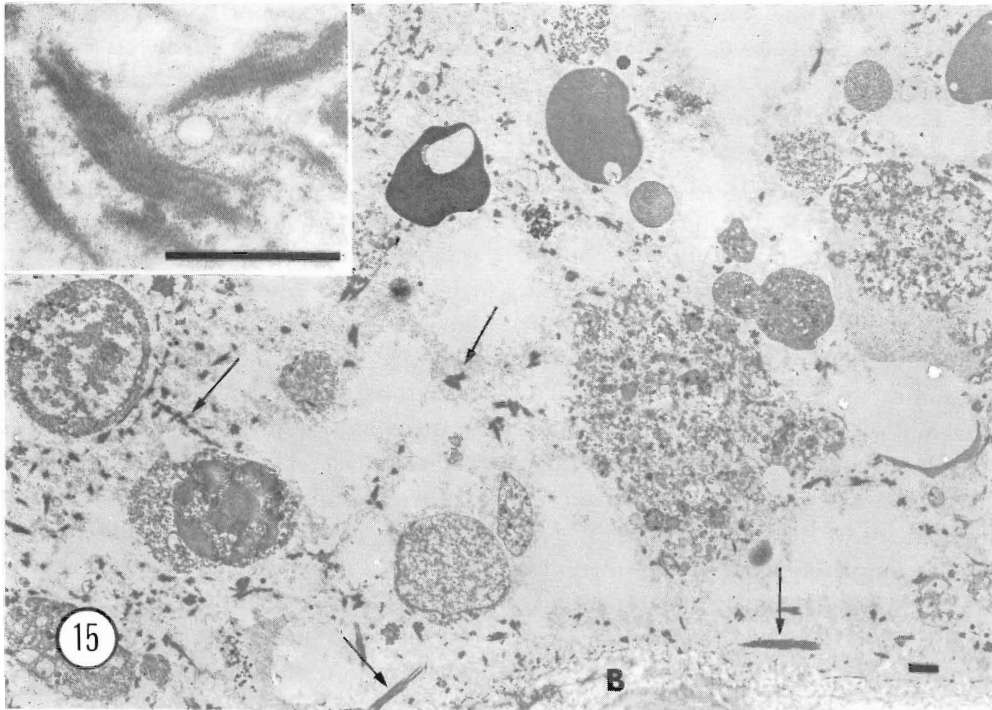
In this study, at 12 and 24 hours after the colchicine injection, the appearance of the fibrils with about 200 Å periodicity was prominent (Fig. 15). They may be the collagen precursor which are derived from the osteoblasts by the destruction of the cell.

As shown in Fig. 14, some osteoblasts seemed to be not so affected by their secretory process. They were found in the same section in which the other osteoblasts showed a remarkable inhibition in the secretory process. This suggests that the inhibitory effects of colchicine on the secretory function depend on the difference of the functional activities of the osteoblasts before the colchicine injection.

It has been shown that the microtubules serve as a cytoskeleton to maintain the cell shape (Langunoff and Chi,⁴⁰ Olmsted and

Fig. 15. Low power electron micrograph of the bone surface at 24 hours after the colchicine injection. Osteoblasts have disappeared and many cell debris and autophagic vacuoles are found in the bone marrow near the bone surface. Bundles of fibrils also appear (arrows). B: bone matrix. Bar, $1.0 \mu \times 2,300$. Inset: higher magnification of fibrils. Periodicity found in the bundles is about 200 Å. Bar, $1.0 \mu \times 23,000$

Fig. 16. Electron micrograph of the cell at 24 hours after the colchicine injection. Scarcity of cytoplasm is noted. Spherical cisterna of the RER (arrow heads) and small vesicles are also noted. Sometimes vacant spaces (asterisks) and destruction of plasma membrane (arrow) are found in the cell. B: bone matrix. Bar, $1 \mu \times 6,000$. Inset: Microtubules (arrow heads) are seen in the cytoplasm occasionally. Bar, $0.5 \mu \times 25,000$



Borisy³⁾). When the microtubules were destroyed, the elongate or elliptical cells were transformed into a more spherical shape. This is compatible with the findings of the present study (Fig. 13).

It is accepted that most of the effects of colchicine are due to the specific binding of this drug to the microtubular subunit, namely tublin (Wilson^{4,5)}). Since colchicine alters the equilibrium between the tublin and the microtubules in the cytoplasm, the microtubules are expected to be depolymerized and the disappearance of the microtubules may occur (Patzelt *et al.*,⁴¹⁾ Fuller *et al.*⁴²⁾). However from the observations in the present study, the microtubules did not disappear completely from the cell after the colchicine injection (Fig. 12, 16). It seemed to be difficult to explain that the changes in the osteoblasts of the colchicine-administered rats are caused only by the depolymerization of the microtubules.

In the present study, the destruction of the plasma membrane was observed (Fig. 12, 16). It has been reported that colchicine binds to the isolated membrane fractions (Stadler and Franke⁴³⁾) and that the tublin was found in the isolated membrane fractions (Stephens⁴⁴⁾). Colchicine seems to affect the distribution of the intermembrane particles (Wunderlich *et al.*⁴⁵⁾) and have various effects on the patching and capping of the cell surface receptors (Yahara and Edelman⁴⁶⁾). Recently Nicolson⁴⁷⁾ suggested that the microtubules as well as the microfilaments might be associated with the plasma membrane and are involved in the transmembrane control of the receptors. As a consequence, the effects of colchicine both on the microtubules and the plasma membrane may be closely related with the inhibition of the secretory process, the destruction of the plasma membrane and finally cell death as shown in the present study.

The appearance of the microfilaments of from 80 to 110 Å in diameter (Fig. 11) is consistent with the observation about the colchicine-treated cell in the previous studies in which these microfilaments have been considered to be protofilaments of the microtubules (Ishikawa *et al.*,⁴⁸⁾ Wisniewsky *et al.*,⁴⁹⁾ Bhisey and Freed,⁵⁰⁾ Holtrop *et al.*,¹⁷⁾ Ehrlich *et al.*¹⁴⁾). Recently, however, De Brabander *et al.*⁵¹⁾ reported that by using the immunocytochemical technique these microfilaments did not reveal the nature of the protofilaments of the microtubules. The origin and the function of these filaments are still not clear.

Heath *et al.*,⁵²⁾ Reaven and Reaven,⁵³⁾ Chanard *et al.*,⁵⁴⁾ Ogura *et al.*⁵⁵⁾ and Okayasu⁵⁶⁾ reported that by using a similar dose of colchicine as in the present study the concentration of the serum or plasma calcium decreased after the colchicine administration. Colchicine was found to block the bone resorption (Raisz *et al.*¹⁶⁾) and to reduce the function of the osteoclasts (Holtrop *et al.*¹⁷⁾). It was proposed that the inhibition of bone resorption might be responsible for the hypocalcemia caused by colchicine (Heath *et al.*⁵²⁾). However, it is very doubtful, from the findings in the present study, that only the inhibition of bone resorption would occur in the colchicine-treated rats. It is well recognized that the bone is an important component of the homeostatic mechanism for maintaining the mineral concentration in the blood plasma within carefully defined limits. The control of calcium homeostasis is thought to be achieved by the bone lining cells named "osteocyte-lining cell bone unit" (Talmage *et al.*⁵⁷⁾) or "bone metabolic unit" (Rasmussen and Bordier⁵⁸⁾). This unit may play as a functional membrane (Davis *et al.*⁵⁹⁾) and control the rate of influx and efflux of calcium from the bone to the extracellular fluid.

It may be possible to consider that the destruction and the inhibition of this unit as shown in the present study might be concerned with the change of calcium homeostasis caused by colchicine.

It is concluded by the results obtained in the present study that colchicine induces a remarkable inhibition of the cellular function of the osteoblasts *in vivo*. The precise mechanism of the action of colchicine on the bone cells, including the osteoclasts and osteocytes, remains to be explained by further studies.

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