DISTRIBUTION AND CONTENT OF MICROTUBULES IN RELATION TO THE TRANSPORT OF LIPID

An Ultrastructural Quantitative Study of the Absorptive Cell of the Small Intestine

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ABSTRACT
To determine whether microtubules are linked to intracellular transport in absorptive cells of the proximal intestine, quantitative ultrastructural studies were carried out in which microtubule distribution and content were determined in cells from fasting and fed animals. Rats were given a 1-h meal of standard chow, and tissue was taken from the mid-jejunum before, 1/2 h, and 6 h after the meal. The microtubule content of apical, Golgi, and basal regions of cells was quantitated by point-counting stereology. The results show (a) that microtubules are localized in intracellular regions of enterocytes (apical and Golgi areas) previously shown to be associated with lipid transport, and (b) that the microtubule content within apical and Golgi regions is significantly (P < 0.01) reduced during transport of foodstuffs. To determine the effect of inhibition of microtubule assembly on transport, colchicine or vinblastine sulfate was administered to postabsorptive rats, and the lipid and microtubule content of enterocytes determined 1 and 3 h later. After treatment with these agents, lipid was found to accumulate in apical regions of the cells; this event was associated with a significant reduction in microtubule content. In conclusion, the regional distribution of microtubules in enterocytes, the decrease in assembled microtubules after a fat-containing meal, and the accumulation of lipid after the administration of antimicrotubule agents suggest that microtubules are related to lipid transport in enterocytes.

It is well known that mucosal epithelial cells of the small intestine are able to absorb and secrete large quantities of lipids and other foodstuffs. Although certain regulatory factors relating to the initial events of absorption and reconstitution of various substances have been identified in enterocytes (2, 6, 10, 16), very little is known of the mechanisms which control the transcellular flow and ultimate secretion of absorbed substances. In view of the suggested role of microtubules in intracellular transport processes in many tissues (3, 8, 9, 13, 17, 19, 22), it seemed possible that these organelles might also be involved in transport events in enterocytes. Indeed, evidence has recently been presented in support of this view by Glickman et al. (5), who indicated that the rate of intestinal lipid secretion was decreased in rats given colchicine. These authors suggested that this effect was
related to the ability of colchicine to prevent microtubule assembly. We have focused on this question more directly by studying the relationship between lipid transport and microtubule content and distribution that occurs in enterocytes in the fasting state, at various times after eating, and after the administration of antimicrotubule agents.

MATERIALS AND METHODS

Experimental Procedure

In meal-feeding experiments, female Sprague-Dawley rats (200 g) were trained over a 6-day period to consume food in a single meal (standard rat chow containing as percent of total calories 10% fat, 60% carbohydrate, and 30% protein) provided between 8 and 10 a.m. each day. After a slight initial weight loss, the animals maintained their weight on this regime and managed to consume 10-15 g of food each meal period. On the morning of the 6th day, food was withheld from some rats (fasting) and provided to others for a 1-h interval (fed). The rats were stunned by a blow to the head, and tissue was removed from the mid-jejunum (22-23 cm from the pylorus) either before, 1/2 h, or 6 h after completion of the 1-h meal. Specimens of intestine were prepared for electron microscopy and examined for content and distribution of lipid and microtubules as well as other cell organelles.

In additional experiments, the effect of colchicine, lumicolchicine, and vinblastine sulfate on lipid accumulation and microtubule content and distribution was determined. Rats were allowed to eat normally, food was removed at 8 a.m. the day of the study, and colchicine (0.05-5.0 mg/kg), lumicolchicine (10 mg/kg), or vinblastine sulfate (10-40 mg/kg) was given intravenously at 11 a.m. 1 or 3 h later, the rats were decapitated, and intestinal tissue was taken (22-23 cm from the pylorus) for electron microscope examination.

Ultrastructural Morphometric Procedures

Excised segments of jejunum were slit open, gently cleansed, and immediately covered with either 2% glutaraldehyde (Polysciences, Inc., Warrington, Pa.) in 0.1 M cacodylate buffer (mosmol = 430; pH 7.2, 22°C) or 1% osmium tetroxide in Millonig's buffer (pH 7.0, 4°C). The segments of intestine were cut into 3-mm widths and submerged in fixative overnight. The following day the glutaraldehyde-fixed samples were postfixed for 1.5 h in 1% osmium tetroxide in Palade's veronal buffer (pH 7.0) and stained en bloc for 1.5 h with 2% uranyl acetate in veronal buffer (pH 5.5). All tissues were subsequently dehydrated in ethanol and embedded in Epon-Araldite plastic. Thick sections were scanned for upright villi, and only those villi containing longitudinally oriented absorptive cells were thin-sectioned and placed on Formvar-coated slot grids and stained by standard techniques. Only superficially located villus cells (i.e., cells located between 2-10 cells of either side of the villus tip) were studied. Occasionally, crypt cells were examined for comparison with villus cells. Apical, Golgi, and basal regions of the first six nucleated cells viewed, which fit the above criteria, were photographed at magnifications (× 16,000) at which microtubules could not be readily identified. When the size of the cell did not permit the entire region to be photographed in a single micrograph, the left-hand edge of the cell, including the lateral plasma membrane, was always chosen. In general, micrographs from the apical cytoplasm excluded microvilli but included the terminal web region and most of the superficial half of the supranuclear portion of the cells; the Golgi area represented the remaining portion of the supranuclear cytoplasm and usually included an entire Golgi complex, the tip of the nucleus, and the lateral region of the cell between the Golgi complex and the plasma membrane. The basal, or infranuclear, region of the cell could generally be photographed in its entirety.

Quantitative estimates of the fraction of the cytoplasmic volume occupied by lipid droplets, microtubules, and various other organelles were obtained by point-counting stereotechnical techniques as outlined by Weibel (20) and used in previous studies from this laboratory (14, 19). In brief, transparent grids with lattice dimensions appropriate to the structures being examined were placed over each photographic enlargement (× 3) of randomly obtained electron micrographs, and the number of points (P) of the lattice that fell on a particular structure, relative to the number that fell on the cytoplasm, was recorded. The value for the fractional volume (volume density) of the structure within the cytoplasm was obtained from the ratio, P structure /P cytoplasm, corrected for the dimensions of particular lattices used. Blindfold techniques were used throughout the study, that is, individuals who photographed the cells and individuals who both labeled and estimated structures on photographic prints did not know the origin of the tissues while this work was in progress.

The estimation of microtubules by the point-counting

2 Lattices in point-counting stereology generally provide a means of obtaining random points that are infinitely small relative to the structures being evaluated (20). In the construction of a millimeter grid with which to estimate microtubule content, it was found that points or line-intersections, which were bold enough to be usable, were relatively large compared to the size of the microtubules themselves. In using such a grid, it was found that microtubule volume density was overestimated by a factor of 2.5. To compensate for this error, all estimates of microtubule volume density recorded in this report have been corrected by a multiplication factor of 0.4.

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method appears to be highly reproducible. In our laboratory, if the same technician is given five identical prints in which to label and count microtubules, the coefficient of variation between microtubule measurements on these prints is <5%. However, statistical analyses of the data suggest that the variation in cell content of microtubules, as estimated in this study, is considerably larger. These combined values are reflected in the size of the standard error of the mean for microtubules reported in this study; thus, the coefficient of variation in microtubule content between intestinal absorptive cells (apical region) of individual fasted rats was found to be 36%, and the coefficient of variation in microtubule content (apical region) between cells of different fasted rats was found to be 10%. Despite these variations, Figs. 4 and 5 and Tables I and II of this study indicate that statistically significant changes in microtubule content between regions of the same cells and between cells from experimentally different animals can be reliably measured.

RESULTS

General Observations

The fine structural details of intestinal cells from fed and fasted animals have been repeatedly described (2, 4, 7, 11, 18) and so will be described here only to relate the location of microtubules to the location of lipid particles.

In cells from fasting animals, the majority of the lipid particles seen were of very low density lipoprotein (VLDL) size (400-600 Å), and were located as individual particles within cisternae of the smooth endoplasmic reticulum (SER) in the subapical portion of the absorptive cell. In most cells, vacuoles located on the periphery of the Golgi complex also contained clusters of particles of VLDL size. Lipoprotein particles were not seen below the Golgi apparatus or in basal regions of the cell. In general, the distribution of microtubules in cells from fasted animals appeared to follow the regional distribution of VLDL, that is, microtubules were seen in the apical portion of the cell (Fig. 1) and around the Golgi complex (Fig. 2), but were rarely observed in the basal portion of the cell (Fig. 3). Although microtubules were occasionally seen in close proximity to certain cell structures, they did not appear to show a regular association with any particular structure. Microtubules were frequently seen in the cytoplasm surrounding a Golgi complex. However, in general, the microtubules did not penetrate the interior of the Golgi complex between the membranous sacs and VLDL-enclosed Golgi vacuoles, nor did there appear to be a special relationship of microtubules to the lateral plasma membrane (Fig. 2).

The most conspicuous change in the morphology of cells of fed animals was the appearance of chylomicra (lipid particles >1,000 Å diameter) within the apical cytoplasm and Golgi vacuoles. In cells from animals that had just completed a 1-h meal, chylomicra were quite prominent in the cisternae of rough endoplasmic reticulum (RER) and SER and in Golgi vacuoles. 6 h after the animals had eaten, chylomicra were found primarily in Golgi vacuoles.

Although the inspection of the postabsorptive cells suggested that there was a diminished number of microtubules in these cells as compared to cells from fasting rats, no particular change in the orientation or distribution of the existing microtubules was apparent. Several of these observations have been subjected to quantitative confirmation and are reported in some detail below.

Microtubule Content in Different Regions of Absorptive Cells

Cells from fasting animals: Fig. 4 indicates that the mean cytoplasmic content of microtubules varies in absorptive cells depending on the region examined. Both apical and Golgi regions contain significantly more (P < 0.01) microtubules than are present in the basal region. In addition, there are slightly more microtubules in the apical region of these cells than in the Golgi region (22%), but this difference is not statistically significant.

Cells from fed animals: Fig. 5 indicates that 1/2 h after eating, the volume density of microtubules decreases significantly (P < 0.01) in both the apical and Golgi regions of absorptive cells. In the apical region, microtubule volume represents only 51% of its volume in fasting cells; in the Golgi regions of the same cells, microtubule volume represents 56% of its volume in fasting cells. 6 h after eating, the microtubule volume in both apical and Golgi regions shows a return toward fasting values. This feature is most noticeable in the apical region, where microtubules now represent 85% of fasting values. Finally, this decrease in microtubule content of apical and Golgi regions cannot be a result of a relocation of microtubules to the basal area of the cell, as the negligible content of microtubules present in this region of the cell (Fig. 4) is unchanged after eating.

Additional morphometric measurements: The results given in Fig. 5 indicate that the microtubule content of apical and Golgi
areas of intestinal cells decreases after a meal. However, it was not immediately clear whether this decrease represented an absolute loss of assembled microtubules, or an apparent loss due to lipid engorgement or cytoplasmic swelling after the absorption of lipids and other food-stuffs. To evaluate these issues, several additional morphometric measurements were carried out on the same cells in which the original microtubule measurements had been made. In the original measurements, microtubule estimates were based on cytoplasm that included cytoplasmic lipid. These values were seen in Fig. 5 and are presented in tabular form in Table I, measurement 1. To deter-
mine whether this amount of lipid influenced values of microtubule volume density, the lipid volume was separately measured and subtracted from calculations of cytoplasmic volume. As shown in Table I, measurement 2, exclusion of lipid from the cytoplasm did not substantially influence microtubule volume as originally reported.

To assess regional swelling, we estimated the volume content of mitochondria in the apical region of the identical prints used in the estimate of microtubules. No decrease in mitochondrial values was seen in cells from fed rats (Table I, measurement 3). Finally, to estimate changes in cell volume more directly, additional low magnification photographs were taken of absorptive cells from fasting, 1/2-h, and 6-h postabsorptive animals. Nuclear/cytoplasmic volume density ratios were obtained from these cells, and, as can be seen in Table I, measurement 4, no difference in this ratio was obtained in fasting versus postabsorptive cells.

As an additional control, measurements were
also made of the microtubule volume density of the apical cytoplasm of crypt cells obtained from the same tissue blocks as the villus cells. These cells would not be expected to undergo postabsorptive changes as they play no role in absorption of foodstuffs. To insure well-fixed cells for this part of the study, only those crypts located on the outside edge of tissue blocks were examined. Although crypt cells obtained in this way appear to have fewer microtubules than villus cells, their microtubule content does not significantly change with eating (Table I, measurement 5).

**Effect of Colchicine and Vinblastine Sulfate on Absorptive Cell Microtubule Content and Lipid Accumulation**

The microtubule content of the apical cytoplasm of absorptive cells decreases after colchicine administration. This decrease in microtubules appears to be related to the dose of colchicine given and the time that elapses after colchicine administration (Table II). Further, this decrease in microtubules is related in a general way to the amount of lipid that accumulates in the apical portion of...
FIGURE 4 Mean (± SE) microtubule content in different regions of intestinal absorptive cells from the jejunum of fasting rats. The mean content of microtubules within apical regions of these cells is 22% higher (not significant) than in Golgi areas, and 86% higher (P < 0.01) than in basal areas. Values were obtained from measurements of three photographs of each of six cells from each of the animals indicated by parentheses in the data bars. Tissue was glutaraldehyde fixed.

The absorptive cells (Table II). It can be seen that very low doses of colchicine (0.05 mg/kg) given for 3 h will reduce microtubules by approximately the same amount as a very high colchicine dose (5 mg/kg) given for only 1 h. The amount of lipid that accumulates in the cells is also similar in both these situations. It should be noted, however, that even very high doses of colchicine (5 mg/kg) given for 3 h will not eliminate all microtubules from the cells. The accumulation of apical lipid in these cells can be quite extensive (see also Fig. 6), however, and the mean value is some seven times that seen in normal cells.

To determine whether the accumulation of lipid in intestinal cells after colchicine administration was related to colchicine in some manner other than through its effect on microtubules, two additional experiments were conducted. In one, rats were given another antimicrotubule agent (vinblastine sulfate) in increasing doses until microtubules in intestinal cells were found to be markedly diminished. In untreated cells of three postabsorptive rats given 40 mg/kg purified vinblastine sulfate (obtained from Dr. R. J. Hosley, Eli Lilly and Company, Indianapolis, Ind.), microtubules were greatly reduced (mean [± SE] microtubule volume density × 10⁴ = 1.55 ± 0.49, as compared to 8.6 ± 0.65 for saline controls) and the lipid accumulation in these cells was found to be similar to that seen in cells after high doses of colchicine (mean [± SE] lipid volume density × 10² = 6.11 ± 3.60 as compared to 1.45 ± 0.55 for saline controls). In the second control study, lumicolchicine (10 mg/kg), an isomer of colchicine that does not bind to microtubule subunit protein (21), was administered intravenously to three postabsor-

FIGURE 5 Mean (± SE) microtubule content of intestinal absorptive cells before, ½ h, and 6 h after a 1-h meal of standard rat chow. Immediately after eating, microtubule content decreases approximately 50% in both apical and Golgi regions (P < 0.01). 6 h after eating, microtubule volume shows a return toward fasting values; in apical regions, microtubules represent 85% of fasting values at this time period, whereas in Golgi regions microtubules represent 63% of fasting values. Values were obtained from measurements of six photographs from the apical region and six photographs from the Golgi region of each animal in each experimental category. Numbers in parentheses are the number of animals examined. Tissue was glutaraldehyde fixed.
**TABLE I**

| Measurements | Number of rats/experimental category | Before eating | 1/2 h after eating | 6 h after eating |
|--------------|--------------------------------------|--------------|-------------------|-----------------|
| 1 Microtubule volume density $\times 10^4$ (lipid included) in apical portion of villus cells | 10 | 9.60 ± 1.00 | 4.90 ± 1.02 | 8.20 ± 1.04 |
| 2 Microtubule volume density $\times 10^4$ (lipid not included) in apical portion of villus cells | 6 | 9.90 ± 1.00 | 5.40 ± 1.01 | - |
| 3 Mitochondrial volume density $\times 10^2$ in apical portion of villus cells | 4 | 9.84 ± 1.32 | 9.45 ± 0.83 | 10.56 ± 0.81 |
| 4 Nuclear/cytoplasmic ratio in villus cells | 8 | 0.26 ± 0.02 | 0.25 ± 0.01 | 0.27 ± 0.02 |
| 5 Microtubule volume density $\times 10^4$ (lipid included) in apical portion of crypt cells | 4 | 4.95 ± 0.60 | 4.55 ± 0.40 | - |

* Values were obtained from six randomly selected cells per animal in each experimental category; thus, in measurement 1, cells were examined from 10 fasted rats and 10 rats 1/2 h, or 6 h after a 1-h meal. Tissue was glutaraldehyde fixed and measurements were made at $\times 48,000$.

**TABLE II**

| Treatment | Time after treatment | Number of rats | Volume density microtubule $\times 10^4$ | Volume density lipid $\times 10^5$ |
|-----------|----------------------|----------------|----------------------------------------|----------------------------------|
| Saline control | 3 | 12 | 7.90 ± 0.51 | 2.02 ± 0.70 |
| Colchicine | 0.05 mg/kg | 6 | 4.90 ± 0.80 | 3.00 ± 0.66 |
| 0.50 mg/kg | 3 | 6 | 1.20 ± 0.50 | 5.12 ± 1.68 |
| 5.00 mg/kg | 6 | 1.10 ± 0.21 | 14.32 ± 3.09 |
| Colchicine | 5.00 mg/kg | 1 | 6 | 3.61 ± 0.40 | 3.60 ± 1.14 |

* All animals had food removed from cages at 8 a.m. Rats given colchicine were injected at 11 a.m.

These alterations (i.e., the intra- and extracellular accumulation of large lipid particles and the displacement of Golgi complexes) are seen also in cells from colchicine-treated animals that have been without food for 24 h. Presumably, even the small amount of lipid absorbed from sloughed cells and recycled bile (7) in the fasted rat is retained in the apical portion of the cell.

To quantitate these observations concerning the colchicine-induced redistribution of lipid and cell organelles, additional morphometric measurements were made on low magnification micrographs of intestinal cells. In photographs of cells with roughly similar dimensions, a line was drawn dividing the supranuclear portion of the cell into...
two parts, a superficial or "subapical" region, and a deeper region regarded as the "nuclear" region. Intracellular and extracellular lipid, as well as stacked SER membranes (considered to be part of the Golgi complex), were outlined on all prints, and their volume densities were estimated by standard point-counting stereological techniques. (Osmium tetroxide-fixed cells were used in this portion of the study to facilitate identification of lipid particles in low-magnification micrographs.) As shown in Table III, the values for intracellular and extracellular lipid and Golgi membranes were greater in the nuclear region than in the subapical region of the control cells, whereas the reverse
FIGURE 7  (a and b) Portions of the apical region of two absorptive cells from rats given colchicine 3 h earlier. Golgi complexes (G) are seen in abnormally superficial locations. Osmium tetroxide fixation. × 36,500.
TABLE III
Distribution of Lipid and Golgi Membranes in Apical Mucosa of Control and Colchicine-Treated Rats

| Treatment*       | Tissue component | Volume density $\times 10^3$ | A  |
|------------------|------------------|-------------------------------|----|
|                  | (A)              | (B)                           |    |
| Control          | Intracellular lipid | 46.50 ± 5.00          | 60 |
|                  | Extracellular lipid | 0.54 ± 0.25           | 54 |
|                  | Golgi membranes\[ | 0.50 ± 0.07            | 50 |
| Colchicine       | Intracellular lipid | 2.00 ± 0.45           | 0.25 |
|                  | Extracellular lipid | 0.75 ± 0.35           | 0.41 |
|                  | Golgi membranes\[ | 0.01 ± 0.00            | 0.03 |

* All animals had food removed from cages at 8 a.m. Rats given colchicine (5 mg/kg body wt) were injected at 11 a.m. and killed 3 h later.
\[ Tissue was osmium tetroxide fixed, measurements were made at \times 17,000 from 6-10 cells obtained from each of five animals.
\[ Mean is ± standard error.
\[ Only stacked membranes of Golgi complexes were included in measurement.

was true in cells from colchicine-treated rats. When the results are expressed as nuclear/subapical ratios (Table III, column 6), values from control animals are always greater than unity and values from colchicine-treated rats are always less than unity, indicating a substantial reorganization of these cellular components in colchicine-treated animals.

DISCUSSION
Special features of the intestinal absorptive cell make it a useful tissue in which to assess the relationship of microtubules to intracellular transport and secretion. Absorptive cells are oriented so as to absorb and/or to synthesize substances at one end and to secrete them at another; as such, different regions of the cell are functionally distinct and the definition of the distribution of organelles within these regions may provide insights as to their function. In addition, transport mechanisms can be initiated in these cells by simply allowing the animals to eat. When the substances being transported are visible with the electron microscope (as in the case with lipids in intestinal absorptive cells), it is possible to monitor the movement of substances through functionally different regions of the cell, and to correlate this transcellular flow with other ultrastructural events. Finally, it is possible to interfere with the function of a given structure (preventing microtubule assembly, for example), and to assess the effect of this maneuver on both transcellular flow of lipid and associated events. We have used all three of these experimental approaches in the current study, and we believe that the results are consistent with the hypothesis that microtubules play a role in lipid transport in enterocytes.

It is now generally accepted that newly synthesized VLDL and chylomicrons migrate from the SER in the apical cytoplasm of the absorptive cell to the Golgi apparatus where they are modified biochemically, repackaged, and carried to the lateral cell surface in vacuoles formed by the Golgi membranes. It is of interest that the vast majority of microtubules in the enterocyte appear to be located in the apical and Golgi regions, and considerably more microtubules are present in the apical regions of absorptive cells than in the apical regions of crypt cells which are thought not to participate in lipid absorption, transport, or secretion. These observations relating to the selective distribution of microtubules in absorptive cells provide evidence for a link between intact microtubules and the intracellular transport of both VLDL and chylomicra. However, it should be noted that although there is a general relationship between the overall distribution of microtubules and the intracellular pathway of lipid movement, there does not appear to be a special relationship of microtubules to the Golgi vacuoles or the lateral plasma membrane at the level of the Golgi apparatus.

Further evidence for the involvement of microtubules in transcellular lipid movement through
plexes are displaced to very superficial positions in the cell (seen also after the use of other microtubules in this process. Thus, Golgi complexes are related in some way to the regional distribution of lipids; (b) that transcellular flow of lipids after eating is associated with a specific decline in microtubule volume in enterocytes; and (c) that agents that prevent the assembly of microtubules in enterocytes appear to induce an abnormal accumulation of intracellular and extracellular lipid. We believe that these findings are all consistent with the idea that microtubules are related in some way to the intracellular transport of lipids in enterocytes. On the other hand, inasmuch as microtubule content decreases as lipid transport is stimulated, it might be argued that microtubules play no role in transcellular lipid transport. Certainly if it is only the presence of intact microtubules that is important in transport processes in these cells, then the loss of microtubules as a result of either colchicine treatment or the act of eating a single meal should have similar effects on lipid transport. It is obvious, however, that the results of the loss of microtubules is quite different in these two situations; in the colchicine-treated animals, microtubule decrease is related to an abnormal accumulation of lipid within the cells, whereas in the absence of colchicine, microtubules decrease in association with an increase in lipid transport. Although in both cases there is a loss of microtubule content in the cells, after colchicine or vinblastine treatment microtubules are presumably prevented from reforming [21] and the assembly process is functionally paralyzed; whereas in the untreated animals, constant renewal (turnover) of microtubules presumably continues. It appears, therefore, that in intestinal cells it is not merely the concentration of the assembled form of microtubules that is important to intracellular transport; the ability of new microtubules to assemble is also of importance.

Such considerations of possible in vivo changes in microtubule assembly/disassembly kinetics...
complicate efforts to relate microtubules to specific events. We (14, 15) and others (12, 22) have provided evidence in the past that the content of the assembled form of cytoplasmic microtubules can increase in several secretory systems in response to physiological changes. Obviously, it is difficult to present a unified model of microtubule function that can account for the apparent paradox of finding both an increase and decrease in microtubule content under conditions thought to be associated with an increase in microtubule function. It is certainly possible that such a discrepancy in results would exist if microtubules did not play the same role in transport and secretory events in all cell systems. Alternatively, the changes in microtubule content that have been described in these various systems may not all be causally related to the functional events in question. Given the relatively primitive state of our knowledge concerning the role of microtubules in transport and secretion, it is not possible to respond to these issues at this time. However, we have tried to document the fact that changes in microtubule content can, and do, occur in enterocytes under controlled experimental conditions. Hopefully, as more data accumulate, the mechanism by which these changes occur and the causal effect of these changes on cell function will be clarified.

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