LIGHT AND ELECTRON MICROSCOPIC OBSERVATION ON DISORDER OF THE RAT TASTE BUD INDUCED BY METHYLMERCURIC CHLORIDE

By

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Introduction

It has so far been indicated by Takeuchi et al (1) that the characteristic changes of Minamata disease are mainly located on the central nervous system. Takeuchi et al (1, 7, 8, 11) and Eto (9) also reported on the changes of peripheral nervous system in human Minamata disease. In addition, detailed reports examined by electron-microscopy were made by Matsumoto et al (4, 5), Kameda (10), Miyakawa et al (6) and Herman et al (14) on the disorders of peripheral nerve of rats which were affected by experimentally caused methylmercury poisoning, and characteristic changes of sensory nerve were pointed out.

Although some pathological changes in general organs other than nervous system had also been indicated (Takeuchi) in Minamata disease, Takeuchi et al have recently found a disorder of isletcells in pancreas which was associated with hyperglycemia in Minamata disease. On the other hand, another disorder for the sense of taste have been clinically noted in the majority of the patients of Minamata disease as reported by Nosaka et al (3). According to their report, approximately 80 % of the patients had complained of their disturbances for taste. Since the cortices of cerebrum of the autopsied cases have been noticed to be affected in Minamata disease, cause of the taste-disturbance might be attributable to some extent to those changes in the central nervous system, and if it is correct, the gustatory center would be associated with the disturbance.

However, from our past examination on changes of the central nervous system, peripheral nerve-endings and the taste buds were supposed to be more responsible for the disorder of the sense of taste than central nervous system, and the present work was planned to examine the taste bud tissue on the effects of an experimentally caused methylmercury poisoning. From our experience in sural nerve biopsy in man with Minamata disease, an electron as well as light microscopical observations were considered to be necessary in order to obtain more accurate findings. Accordingly, both these techniques were applied for the present work.

Materials and Methods

Wister rats weighing approximately 100g were used as materials. Three mg per day of methylmercuric chloride (mercury content 2.4mg/day) was mixed up with margarine and was given to the experimental animals by oral administration every day or on intermittent days. Control rats were treated in the same way as the experiments except giving margarine without mercury.

For animals for the light microscopical examination, total dose of the mercury content 7 mg (A), 26 mg (B), 55 mg (C, D, E) and 58 mg (F) were given respectively to the 6 animals, although these doses were too much for poisoning. Most of the experimental rats showed clear nervous signs for the disease at the time of about 40 mg of total mercury administration. The tongues of died or killed experimental animals were filled in 10% formalin and prepared for light microscopical sections by the routine technique.

Other four animals for electron microscopical observation (No. 1 to No. 4) were treated separately but in the same way as those for light microscopical examination. No. 1 was given 2.4 mg/day of mercury every day for 6 days (total 14.4 mg). No. 2 was given the same dose every day (total 58 mg). No. 3 was treated by intermittent administration by stopping it when loss of weight was noticed. The onset of the disease of No. 3 appeared on the 25th day of mercury administration. The rat was killed 16 days after the first onset and the total dose was 43 mg. No. 4 was treated intermittently in the same manner as in No. 3. The administration was stopped on the 87th day. Then the animal was killed 16 days after the stop. This rat showed crossing over of the hindlimbs on the 37th day. The total dose of mercury administered was calculated to be 77 mg.

Preparation of the specimens for electron microscopy was carried out according to Kitamuura's technique (13). Animals were anesthetized with ether for 5 minutes and 0.2 ml per 100g of hexobarbital sodium was injected into the back muscle. Abdominal cavity was opened 10 minutes after the injection, the aorta and jugular veins were exposed. Blood stream of the aorta was stopped by ligature at a few millimeters central portion from the branching point of the renal arteries. Then an appropriate part of the aorta in the cardiac side from the ligature was incised. About 30 ml of 3% glutaraldehyde (0.05M cacodylate buffered pH 7.4) was infused through the incised lip of the aorta, retrogradically against the direction of blood stream. When the exposed jugular veins were observed expanded by the infused glutaraldehyde, both of the jugular veins were incised by a sharp razor blade. The infusion of glutaraldehyde from a syringe was continued until tissue fixation of the head and upper extremities became sufficient. The tongue was cut out of the animal body and the tiny pieces of vallate papillae were selectively gathered for electron microscopic examination. The tiny pieces were fixed in the same glutaraldehyde solution in small glass vials for 1 hour further, then postfixated with 1% osmium tetroxide buffered with 0.1M veronal

acetate buffer pH 7.4 for 1.5 to 2 hours. Dehydration and embedding with Epon 812 were done in the routine manner. One micron sections were cut from the embedded tissues before ultrasectioning on the LKB type 1 ultramicrotome. They were stained with toluidine blue and observed under the light microscope for the purpose of identification of necessary areas. After trimming down of the tissue block surfaces, ultrathin sections were made and double-stained with uranyl acetate and leadcitrate (Raynolds). The electron microscope used for observation and photography was a Hitachi HU 11A.

Table 1. Doses of Methylmercury Administered and Experimental Course

Animals	Administration	Onset of symptoms, mg of MeHg & (Hg)	Total clinical course	Total doses	
A	every day	—	5 day	MeHg 15 mg	Hg 7 mg
B	every day	9th day 27 mg (21)	12	36	26
C	every day	16th day 48 mg (38)	23	69	55
D	every day	22nd day 66 mg (53)	23	69	55
E	every day	21st day 63 mg (50)	23	69	55
F	every day	16th day 48 mg (38)	24	72	58
No. 1.	every day	— 18 mg (14)	6	18	14
No. 2.	every day	16th day 42 mg (33)	24	72	58
No. 3.	intermittent	25th day 48 mg (38)	41	54	43
No. 4.	intermittent	37th day 75 mg (60)	103	94	77

Results

1. Light Microscopic Observation

Clinical observations

In Figure 1 the course of methylmercury poisoning in terms of weight changes and neurologic signs is revealed. Animals gained weight more slowly than controls at an early stage and thereafter they gradually lost their initial body weight. Exaggerated adduction (flexion) with crossing over of the hindlimbs when the rat was suspended by the tail, which is one of the three main symptoms (Takeuchi et al), appeared 9 days after the administration in the most clear case. Progressive ataxia followed hindlimbs spaced widely during ambulation and hindlimbs were frequently dragged in ambulation. Abnormal movements such as twitching, circling fusciculations and etc. were not observed, except for tremor in occasion. Motor strength, tested grossly by

withdrawal response, was not appreciably diminished.

A (Total dose for oral administration 7 mg) : The epithelia and lamina propria were observed to be atrophied slightly compared with those of the control tissues (Figs. 2, 5) and it resulted in widening of the interpapillary grooves. There were swelling, vacuolar degeneration and karyolysis of the nuclei of the taste bud cells causing a general appearance of uniformity of the unclear size. Nerve fibers in the lamina propria were faintly degenerative and swelling of Schwann cells was seen. Also the epithelial cells of glands of Ebner in lamina muscularis were vacuolarly degenerative.

B (Total dose 26 mg) : Although no special changes were observed in tongue epithelium, the taste bud cells appeared atrophic and the grooves were slightly widened. Distinct was unevenness in size of the nuclei of the taste bud cells due to vacuolar degeneration and granular condensation of chromatin. Also the cytoplasm of these cells were seen to be occasionally in lytic process. Stromal tissues appeared to be less compact than normal. Some of the nerve fibers in lamina propria were degenerative and the number of Schwann cells was observed inclining to proliferation to an extent. Parts of the mucous and serous gland cells showed slight degenerations.

C (Total dose 55 mg) : Degenerative atrophy of the oral epithelium covering the taste buds appeared and widening of the interpapillary grooves was enhanced. In the taste bud cells, numbers of small nuclei increased in the areas because of condensation of the nuclear chromatin. In contrast with this reduced nuclei, their cytoplasm were markedly swollen. Lamina propria was also atrophic and a slight increase of the numbers of cell-nuclei was observed therein. No special changes of nerve fibers were there in the upper and lower propria except very faint degenerations in part. However, both mucous and serous gland cells appeared in parts obviously degenerative with cytolytic changes.

D (Total dose 55 mg) : Although considerable atrophy was there in the oral epithelium of taste bud areas, the cells in the taste buds themselves were more dominantly degenerative and they appeared markedly pyknotic or almost being exhausted with prominent cytolytic changes. Accordingly, the inside of the degenerating taste buds gave a general appearance of sparse and translucent images under the microscope. Fat tissues were observed increasing in lamina propria and the nerve fibers and the nuclei

Fig 1. Changes of the body weight in experimental (C and No.4) and control animals. Arrows indicate onset of the symptoms. The two experimental rats (C & No.4) were presented in this figure as the representative cases of all the animals examined.

of Schwann cells appeared degenerative. Although normal structures of the nerve fibers in basal areas of lamina propria still remained, the nuclei of Schwann cells appeared to be similarly degenerative as those in the taste buds (Figs. 3, 4). Swelling and cytolytic changes of the mucous and serous gland cells were considerable.

E (Total dose 55 mg) : Atrophy of the oral epithelium in covering or near the taste bud areas had become remarkable and keratinization of the squamous cells is distinctive at the surface of the stratified squamous epithelia. Atrophy of the taste buds were more remarkable than the oral epithelium, and consequently in some areas even gaps between those taste buds and the oral epithelial strata were formed. Most of the nuclei of taste bud cells showed distinctly pyknotic and karyolytic appearances (Figs. 4, 7). Both mucous and serous gland cells revealed signs of cytolytic disappearance in parts.

F (Total dose 58 mg) : Slight atrophy was there in the oral epithelium near taste bud areas. Irregularity in size was seen in the nuclei of taste bud cells with some cytoplasmic swelling. Stromal tissues appeared atrophic in upper lamina propria but edematous in deeper areas. Myelin sheaths of nerve fibers seen in basal areas of lamina propria were swollen. Degenerative changes of mucous and serous gland epithelia were considerably seen.

All these light microscopic findings were summarized in Table 2. Pathological changes of the taste buds were found to be gradually enhancing as dosage of methylmercury induction increased. Results of counting the numbers of nuclei within individual taste buds indicated significant decrease of the numbers in experimental animals in comparison with those of the control rat (Table 3).

Table 2. Pathological Changes Observed in Tongue Tissues

Disorders	Animals	A	B	C	D	E	F	Control
Mucous epithelia	Atrophy	(+)	+	++	++	+++	+	—
	Hyperkeratosis	—	—	+	(+)	++	+	—
Taste bud	Decrease of No. of taste bud	+	+	++	++	+++	+	—
	Nuclear deg.	+	++	+++	+++	+++	++	—
	Irregularity of nuclear size	+	++	+++	++	+++	+++	—
Subepithelial tissues	Atrophy	+	+	++	+	+++	++	—
	Deg. of nerve fibers	(+)	+	++	++	++	++	—
Secretory glands	Increase of fat	—	—	—	+++	—	—	—
	Deg. of mucous gland epithelia	(+)	+	+++	++	+++	+++	—
	Deg. of serous gland epithelia	—	(+)	++	++	+++	++	—
Cell infiltration		—	—	—	—	++	—	—

Table 3. Average Number of Nuclei in Single Taste Buds

No. \ Animals	A	B	C	D	E	F	Control
No. of taste bud counted	28	21	21	22	16	26	27
No. of nuclei	254	228	177	168	86	195	271
Average number of nuclei	9	11	8	7	5	7	10

2. Electron Microscopic Observation

1) Epon-embedded, toluidine-blue-stained, 1 micron cut sections

In the control rat (Fig. 8), borders of the individual cells in each taste bud were clearly recognized. However, in an experimental rat No. 2 (total dose 58 mg, Fig. 9), all the nuclei of the taste bud cells were observed to be decreased their staining intensities with swollen appearances. Differences of the intensities between individual nuclei were being lost and all of them were giving rather uniform image. Similar changes as stated in the light microscopic findings were generally observed in the Epon-embedded, toluidine-blue-stained sections. The pathological changes enhanced as total doses of methylmercury administered increased. These areas of the taste buds were cut for ultrathin sections, observed on the electron microscope and photographed.

2) Ultrastructure of the taste buds of the experimental animals

In an early stage (No. 1. total dose 14 mg, 6th day of administration), although no characteristic changes were manifested both in type 1 (light cell) and type 2 (dark cell) cells, the intercellular spaces were seen widened between neighbouring cell borders forming some crevices and the nuclei of individual cells tended to increase their densities. Therefore, it became a little harder to differentiate these two kinds of cells on photographs (Fig. 11). Microvilli protruding from these cell surfaces were slightly changing their patterns of normal arrangement (Fig. 12). Parts of the mitochondrial matrices were irregularly decreasing their densities causing translucent images. Same kind of changes were seen in mitochondria within nerve axons in this stage, but no special change was there in neurotubules.

As doses of mercury administration increased (No. 2 and No. 3), type 2 cells decreased in number and type 1 cells formed main cellular components in the taste buds. These cells increased their nuclear densities giving more pathological appearances than the squamous epithelial cells surrounding the taste buds. Changes of the mitochondria of type 2 cells were further intensified with noticeable disappearance of the cristae and frequent formation of worm-eating spot-like lesions of the matrices. These changes developed to apparent vacuolarization thereafter, and finally large vacuoles which are merely enveloped with the outer mitochondrial membranes could be seen scattered in the cytoplasm (Figs. 13, 14). In endoplasmic reticula and Golgi areas,

Fig. 2

Control picture of taste buds. Low magnification light-microscopy. The taste buds are even in size and their arrangement in epithelial layer is in order.

Fig. 3 D (23rd day)

Taste buds are affected by methylmercury poisoning. Some of them are evidently atrophic with decrease of number of the cell nuclei.

Fig. 4 E (25th day)

Degenerative changes of the taste buds are more conspicuous than D. Slight atrophy of the surrounding squamous epithelial cells can also be seen with increasing keratinization in surface layer.

Fig. 5

High power light-microscopic appearance of normal taste buds (control animal). The taste bud cells appear to be evenly distributed in each taste bud both in their size and number.

Fig. 6 D (23rd day)

Decrease of number of the taste bud cells are conspicuous in each taste bud, resulting an atrophy of the whole taste bud structures. Intensive is pyknosis of the nuclei and vacuolar degeneration of the cytoplasm in most surviving cells.

Fig. 7 E (25th day)

High power light-microscopy of Fig.4. Unevenness of size and density of the taste bud cell-nuclei are prominent. Their irregular arrangement are also noticeable.

Fig. 8

An Epon-embedded, 1 micron cut and toluidine-blue-stained section of a control animal. Type 1 (light) and type 2 (dark) cells in the taste buds are differentiable under the light-microscopic picture.

Fig. 9

1 micron Epon-section of the No. 2 animal (25th day), toluidine-blue-stained. Degenerative atrophy is there in the taste buds. Type 1 and type 2 cells have become undifferentiable because of their closer densities. Translucent image of the nuclei of the taste bud cells and indistinctness of the cell-border compose degenerative profiles of the cells.

Fig. 10 No. 4 (103 day)

An electron microscopic and general view of about four-fifth of a taste bud. Various changes of organelles of the taste bud cells are shown in this picture such as pyknosis of nuclei as revealed with increase of density, vacuolarization of mitochondria and smooth ER, detouching of ribosomes from ER-membranes, irregular arrangement of Golgi element and abnormal forms of dense bodies. 7,500 ×

Fig. 11 No. 1 (6th day)

Basal area of a taste bud. The intercellular spaces are widened forming abnormal crevice, and the nuclei of taste bud cells are increasing their densities. But the basement membrane of the taste bud preserves intact appearances. 5,000 ×

Fig. 12

Enlarged picture of No. 1. Slight but irregular decrease of densities are visible in areas of mitochondrial matrices of the taste bud cells. No special change is seen in the neurite except for translucent lesion of the mitochondrial matrices affected in the same way as in those of the taste bud cells. 25,000 ×

Fig. 13 and Fig. 14 No. 2 (24th day)

The worm-eating spot-like, translucent lesions in the mitochondrial matrices and loss of cristae are striking in these pictures. In the most severely affected mitochondria, the whole structures are completely vacuolarized. Detouching of ribosomes from rough-surfaced ER membranes and small vacuolarization of smooth ER are the additional changes of the cytoplasmic organelles. 25,000 ×

Fig. 15 No. 3 (41st day)

A part of a taste bud cell. All the mitochondria and a part of endoplasmic reticulum are vacuolarly swollen suggesting functional disorder of the cell. 25,000 ×

Fig. 16 No 2 (24th day)

A neurite is placed between two taste bud cells. Most mitochondria and some ER of the taste bud cells are intensely vacuolarized whereas mitochondria of the neurite still remain in early stage of degeneration. 25,000 ×

Fig. 17

Enlarged picture of a taste bud cell of No. 4. Various sizes of vacuoles are formed by vacuolarization of mitochondria, smooth ER and Golgi elements. The nuclei show pyknotic and condensed appearances. 18,000 ×

swelling of the tubules of smooth-surfaced endoplasmic reticulum and enlargement of the Golgi vacuoles appeared. Microvilli enhanced their irregularity in arrangement. The crevices between each cell widened. Basement membranes of the taste buds remained mostly in normal appearance though observed very faint irregularity of the filamentous arrangement. In the nerve process, the same disorders of mitochondrial structure in axons were observed as described in those of the taste bud cells, but no special changes were there in neurotubules and matrices.

When additional doses of methylmercury were cumulatively given to the animals and in consequence the experimental period prolonged (No. 4), whole structure of the taste buds underwent remarkable changes. It became quite difficult to differentiate type 1 cells from type 2 cells because of highly increasing densities of the nuclei and cytoplasmic changes. Vacuolarization of the mitochondria was also striking. The nuclei of the type 2 cells were highly pyknotic, and in addition parallel structure of double membranes of the nuclear envelopes had been lost forming some irregular intramembranous gaps between the two membranes. In the cytoplasm, there were vacuolarization of mitochondria due to disappearance of cristae and a degenerative change toward translucent matrices. These vacuoles from mitochondrial vacuolarization seemed to have gradually enlarged singly or fused to each other developing to larger vacuoles. Occasional rupture of some of these vacuoles could be also encountered. Thus, a particular and general view of the cells were being produced in areas involving whole taste buds (Fig. 10). These pathological changes of the taste bud cells were quite standing out in contrast with lesser changes of the surrounding squamous epithelial cells. Slight vacuolarization of smooth-surfaced endoplasmic reticulum developed in the cytoplasm and regularity of arrangement of ribosome particles on the rough-surfaced endoplasmic reticulum membranes had been lost. Disorganization of each Golgi element in Golgi complexes was also one of the conspicuous changes of cytoplasmic organelles (Fig. 17). Lysosomes of dense body type appeared rather normal in structure but were increasing in number. Some of the taste bud cells which underwent the most severe affections on their cellular organelles seemed to be in the process close to a cell destruction.

In the neurites, vacuolar degeneration of the mitochondria could be clearly observed and the neurotubules became less noticeable.

Discussion

It should be noted to begin with, that in our experiment excessive doses of methylmercury were generally administered to each experimental rat. Thus, total given dose of mercury amounted to 7 to 77 mg per body.

This was because the mercuric agent was given to the animals by oral administration and some amount of the given doses was estimated to be lost by the animal's refusing action in the course of administration. Secondly, the present experiment was originally

planned to investigate whether or not methylmercury can cause morphological disorders in taste bud which could explain pathologically a taste disturbance complained by patients of Minamata disease. Focus was essentially to clarify the pathogenesis rather than to describe grade of cellular degeneration due to doses or time course. Accordingly, if injection is employed for the mercury administration, lesser amount of it should be given per body.

It had been clinically indicated by Nosaka et al that in Minamata disease, there existed disturbances of the sense of taste as well as of smell. However, the mechanism of origination of the taste-disturbance had not been clarified. Although there are so-called thinning-out decrease and loss of nerve cells in the cortices of cerebrum in Minamata disease, through disappearance of them are rather rare except severe cases, and major nerve cells still survive and remain in the tissues in most cases. Therefore, in order to clarify the mechanism of the taste-disturbance, it was considered to be necessary to investigate peripheral tissues in addition to the central nervous system. Standing on above viewpoint, the present work as an experimental Minamata disease for rat was carried out to find the peripheral factors for the taste-disturbance expecting the taste bud as an affected organ.

The three main symptoms, namely intense flexion of hind legs, abnormal movement and ataxic gait for Minamata disease for rat which has been defined by Takeuchi et al(1) was rendered to confirmation of the disorder for our experimental animals. The symptoms appeared when the mercury administration amounted to minimum 21 mg per body to maximum 60 mg per body respectively. In case of successive administration, the animal showed the onset of disease at the time of 38 mg per body. The average period of administration until onset was 2 to 3 weeks with a case of minimum 9 days and another case of maximum over 1 month. The administration was continued thereafter and the total dose of mercury amounted minimum 26 mg and maximum 77 mg per body. One experimental animal induced by mercury of 7 mg per body for 5 days was used as a case in presymptomatic stage.

In summarizing the light microscopical findings of the taste buds of the rats which were administered methylmercuric chloride, apparent changes of the taste buds were confirmed to have begun in rather early stages of the mercury poisoning. Namely, stainability of the nuclei of the taste bud cells reduced on the 5th day of administration. The nuclear change was accompanied by swelling of the cytoplasm. On the 12th day, cell-degenerative changes such as pyknosis and partial karyolysis of the nuclei, swelling and vacuolarization of the cytoplasm became clearer. At the time of 3 weeks, more complete karyolysis and karyorrhexis appeared in those cells. Cytoplasmic depletion due to extreme swelling composed one of the severe morphological changes in this stage. In the end stage of affection, the dead taste bud cells disappeared and the total cells in single taste bud was counted decreased in number. Most surviving cells in the taste buds also diminished their size because of severe degenerative atrophy in the terminal

stage. Thus, the taste buds themselves revealed atrophic profiles in general appearance and furthermore, even occasional disappearance of whole structure of the taste buds were recognizable.

In contrast with the severe affections by methylmercury poisoning on the taste buds, no distinct changes were found on the squamous epithelial cells surrounding the taste buds, though slight vacuolar degeneration and atrophy were detectable. However, cornification of the cells in surface layers was considerable in some cases.

Swelling of axons of peripheral nerve fibers in lamina propria was visible only by Klüver-Barrera stain whereas no differentiable changes were demonstrated by routine hematoxylin and eosin stain. Effects on Schwann cells were uncertain light-microscopically.

Although no special findings were there generally in salivary gland, the glandular cells were found, in part, in degenerating process. But, further investigation on this problem will be necessary in the future.

On electron microscopical observation, changes of taste buds and neurites in nerve endings became clearer. In the taste buds, apparent changes appeared on the 5th day and these changes developed to severe affections as the experimental stages proceeded. Type 1 cells (light cell) lost their differentiable and characteristic appearance from type 2 cells (dark cell) because of general increase of density of all these cell nuclei. In the cytoplasm, mitochondrial matrices of the taste bud cells decreased partially their density producing worm-eating spot-like lesions. Loss of mitochondrial cristae occurred at the same time and vacuolarization of the whole mitochondria was finally formed. These vacuolarizations developed further by increasing their size and some of them ruptured in the end stage. In endoplasmic reticulum, edge parts of the smooth-surfaced ER were first swollen and it developed to vacuolar size and appearance. Membrane-associated ribosomes were irregularly detached from the rough-surfaced ER membranes. Also free ribosomes lost their normal distributions in the cytoplasm and decreased in number. Arrangement of the Golgi elements took abnormal patterns with irregular arrays of cisternae and uncommon enlargement of the vacuoles. Nuclei increased their densities in initial stage, changing in later stage to an uniform structure. Parallel array of the double nuclear membranes was disorganized by losing or dissociating to each other forming wider intramembranous spaces. Revealing these various ultramorphological changes of organelles described above, the taste bud cells took a course toward cell destruction.

The squamous epithelial cells around the taste buds were relatively resistant to the mercury poisoning. But, similar kind of changes which began with vacuolarization of mitochondria were caused, though less severely, like in the taste bud cells.

Normal appearance of neurites of nerve endings was relatively well-preserved during experiment. However, as the disorder of taste bud developed, difficulty enhanced to recognize them under the microscope. Vacuolarization of mitochondria in axons, the

same change as observed in those of the taste bud cells was the only disorder visible in early experimental stage in the nerve fibers. But in later stage, changes of neurotubules appeared and swelling of the whole neurites joined the main alterations. Affections on tissue areas other than the taste buds were generally slight. Although some mitochondria of the squamous epithelial cells showed worm-eating spot-like lesions, there still remained many normal-appearing mitochondria in the cytoplasm.

From our findings in the present experiment, it has become clear that some confirmable changes could be caused by methylmercury poisoning on the taste buds which are the receptor apparatus for the sense of taste. In addition, the taste bud cells, especially the type 2 cells were demonstrated to be affected most severely among the composition of the taste buds. The disorder began with a special change of mitochondria and developed to the final cell-destruction resulting in decrease of the total number of cells in the taste buds. From these severe morphological changes in taste bud cells, it was easy to speculate that the functional role of the taste buds of the mercury-induced rats would have mostly been lost in the end stage of affections.

It has been well known that there exist sensory disturbances in Minamata disease. But less cumulative knowledge has been obtained on the effects to receptor apparatus. No particular changes by methylmercury has been described on the photoreceptor cells in retina nor stated on the Corti organ. These sensory organs have so far been regarded as being not or less effectively poisoned. However, our results in the present work would urge further investigation on the effects by methylmercury poisoning in sensory receptor apparatus when one knows such severe affections in the taste buds as demonstrated in this experiment.

Summary

Organic mercury poisoning was caused in rats by inducing methylmercuric chloride with per os administration. Pathological changes of the sensory receptor-apparatus for taste were investigated both light and electron microscopically and the following results were obtained.

1. The methylmercury induced in rats caused disorders of the taste buds as a sensory receptor apparatus for taste. The most characteristic changes were observed on the taste bud cells in which severe exhaustion due to cell degeneration occurred resulting a consequential atrophy of the taste buds.

2. Light microscopically, the cells revealed their degenerative appearances such as pyknosis, karyolysis, karyorrhexis and swelling of the cytoplasm.

3. Electron microscopically, it became difficult to differentiate type 1 cells (light cell) from type 2 cells (dark cell), because the difference of density between the two kinds of cells decreased during the degenerative course. In the taste bud cell cytoplasm, a particular change of mitochondrial matrices appeared revealing a worm-eating spot-like, translucent lesions. These lesions developed in later stage to

vacuolarization of the mitochondria with an additional change of loss of cristae. Similar vacuolarization occurred in endoplasmic reticulum and Golgi element. Therefore, whole cytoplasm was observed being occupied by vacuolated organelles in the end stadium. In contrast, the nuclei of the taste bud cells increased remarkably their densities which were regarded to correspond with pyknosis in the light microscopic changes. Some of the severely affected cells were supposed to come to final cell destruction.

4. The same kind of mitochondrial degeneration as that in the taste bud cells appeared also in the nerve endings. Changes of neurotubules came thereafter.

5. No severe affections were found on the squamous epithelial cells surrounding the taste buds. But slight vacuolarization was detectable in some of the epithelial cell mitochondria. So that, the degenerative appearance of the taste buds formed a sharp contrast with intact views of the surrounding tissues.

We understand that our findings of the disturbance of the taste buds which was caused by methylmercury are important because our results found in this experiment are not only the first presentation of pathological changes which can explain the mechanism of taste disturbance in human Minamata disease, but it will suggest a direction of future investigation for other sensory disturbances in methylmercury poisoning.

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塩化メチル水銀によるネズミの味蕾障害についての 光学顕微鏡的ならびに電子顕微鏡的研究

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水俣病患者の中には味覚障害を訴えるものが多数（約80%）あることが野坂らにより指摘されている。しかし、その病理発生に関しては中枢性であるかあるいは末梢性であるかは、なお明らかにされていない。そこで著者は末梢味覚受容器におけるメチル水銀の障害について検討を加えるため、ネズミを使用し、その味蕾におけるメチル水銀の影響を実験的に光学および電子顕微鏡的形態学的に観察した。実験動物は100g程度のwistar系白ネズミを使用し、塩化メチル水銀3mg/日をマーガリンに混じて連日ないし間歇的に経口投与した。ネズミにおける水俣病の三主徴として武内らに決められた後肢交叉、異常運動、失調を臨床症状の目安として、それら動物について光顕的および電顕的に検索した。

実験成績：メチル水銀は味覚受容器の味蕾に障害をおよぼし、構成細胞の変性崩壊を招来し、その数は著しく減少する。光学顕微鏡的には、投与の比較的早期から味蕾細胞は核のピクノーゼ、核崩壊、核融解などの変化がみられ、障害の著しい細胞は消失する。電顕的には、先ず細胞質の電子密度の変化からⅠ型細胞（明細胞）とⅡ型細胞（暗細胞）の区別が困難となり、比較的早期に糸粒体基質の虫喰状透明化が現われ、次いで内臓が消失し、リボゾームの消失などがあって、最後に糸粒体全体が空胞化したのち崩壊する。小胞体やGolgi装置にも変化が現われ、内腔の拡張膨化を来すため、細胞質全体がこれらの空胞で占められるに至る。他方、核は電子密度の増加と変形核質の凝集など光顕所見に合致した変化を示し、かくして細胞全体の著明な変性像の後崩壊する。神経終末においてはリボゾームの消失、基質の膨化などがみられるが糸粒体の変化は著明に軽く、味蕾細胞と同様な空胞性変化が軽度のみられる。進行期には神経細管も認め難くなる。味蕾周囲の口腔扁平上皮には著変がないが、後になって糸粒体の空胞化がみられるが、細胞障害像は味蕾細胞に比べると著しく軽い。

今回の実験からメチル水銀は味覚受容器としての味蕾の細胞に重大な障害を与えうることが明らかとなった。このことはヒトの水俣病における味覚障害の病理発生についての重要な知見であると共に、その他の感覚障害についても今後の研究に重要な示唆を与えるものと考えられる。