propriate as the cause of the present teratogenic effects of MNU, since they cannot explain why there is a critical gestational day of MNU treatment for the production of congenital malformations; this is why the treatment with 10 mg/kg MNU of pregnant mice on gestational days 0.5 and 1.5 resulted in no malformed fetuses, though the same treatment after gestational day 2.5 resulted in statistically significant rises in the mean malformation rates.

In preimplantation development of mice, it is considered that the metabolic activities of the embryos before the early 2-cell stage are almost entirely controlled by the maternal information which has been stored in the eggs in the form of various RNAs and proteins[17,18]. The expression of the embryonic genome appears to start at the late 2-cell stage and thereafter the embryonic development rapidly falls under the control of the embryonic genome[17,18]. Since the mouse embryos on gestational day 1.5 are mostly at the late 2-cell stage and those on gestational day 2.5 are at the 8-cell stage, one of the reasons why there is a critical gestational day for the production of congenital malformations by the MNU treatment may well be closely related to the differences in the degree of expression of the embryonic genome in mouse embryos on different gestational days. MNU may only slightly influence the inactivated embryonic genome, but can affect the highly activated embryonic genome and cause a certain genetic imbalance which may be expressed later in the oranogenetic period of embryonic development.

Alternatively, MNU may have some toxic effects on the embryos in process of implantation, causing the delayed implantation and the subsequent non-specific overall retardation of fetal growth. It is possible that some malformations may result secondarily from such an overall growth retardation during fetal development. The precise mechanism of MNU teratogenicity on pregnant mice before implantation must await further elucidation.

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Surface morphological study of Ehrlich ascites tumor cells exposed to microwave irradiation and heat[1]

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Summary. Microwave irradiation of EAT cells caused an increase in length and number of surface microvilli. The tumor cells tend to form large aggregates by means of extensive interdigitation of surface microvilli. On the other hand, heat hyperthermia caused a decrease of surface microvilli but an increase of surface blebs. Hence the surface morphology of EAT cells after in vitro exposure to microwave irradiation differs markedly from that after heat hyperthermia.

Recent experimental and clinical investigations have confirmed previous observations that moderate hyperthermia treatment may inhibit the growth of, or destroy malignant tissues[2-8]. Light microscopic studies of various experimental tumors heated within the range of 41–43 °C have shown that cell destruction occurs specifically in malignant cells without damage to normal cells, such as fibroblasts and endothelial cells[3]. These findings have been confirmed by electron microscopic observations[5,9]. In addition, a similar difference has also been reported in tissue culture[10-12]. Chen and Heidelberger[13] discovered that transformed mouse prostatic cells have a much higher sensitivity to heat at 43 °C than normal cells. Kase and Hahn[14,15] found that at 43 °C the heat sensitivity of a virus-transformed human fibroblast line was pronounced. In connection with these studies, Westra and Dewey[16] discovered that mammalian cells in division are more sensitive to being killed by heat particularly cells in the S or G_1 phase of the cell cycle. They reported that when Chinese hamster ovary cells were heated in a 45.5 °C water bath for 7–11 min, more than 90% of the cells at the next division were tetraploid. Coss et al.[17] revealed that heat completely dissembled the intact microtubules and inactivated a proportion of the the microtubular proteins in vitro. In spite of the attempts made by previous investigators, the mechanism by which tumor cells are inhibited and

Table 1. Number and length of microvilli before and after microwave treatment

Microwave treatment	Microvilli Number/μm²	Length/μm
Control untreated EA cells	11.7 ± 8.6*	0.78 ± 0.41
15 min	24.5 ± 7.3	1.27 ± 0.09
25 min	39.4 ± 13.1	1.30 ± 0.16

Table 2. t Value of different experimental conditions

t Value	Microvilli Number/μm²	Length/μm
Control vs 15 min treatment	3.5799*	4.0238*
Control vs 25 min treatment	5.5966*	4.0088*
15 min treatment vs 25 min treatment	3.1444*	0.4102

*Significant at 15%.

destroyed by hyperthermia remains unknown. In this communication, we report the effect of microwave irradiation on Ehrlich ascites tumor cells in vitro, observed by scanning electron microscopy. In particular, this effect was compared to the changes of surface morphology caused by heat hyperthermia.

Materials and methods. The Ehrlich ascites tumor (EAT) cells have been carried by intraperitoneal transplantations in mice in our laboratory for about 10 years. The ascitic fluid containing EAT cells was collected from tumor-bearing mice and kept at room temperature (23 °C) before treatment. Apart from the control untreated group, some specimens were irradiated with a Minato Microtizer (Model MT-150, 2450 MHz) at 120 W for 15 and 25 min. After treatment, the temperatures were 34 and 36 °C respectively. Other samples were incubated in water baths at 41 and 46 °C for 30 min. Thereafter, all the specimens were fixed in 2.5% glutaraldehyde in cacodylate buffer for 1 h. This was followed by dehydration, critical point drying (Ladd Research Industries) and coating with gold (Edwards Sputter Coater, S-150B). The coated specimens were examined with a Jeol JSM-35CF scanning electron microscope at 15 kV.

Results. In the control untreated group, the EAT tumor cells were either single or formed small aggregates (fig. 1). The tumor cells were usually spherical in shape and varied in size, averaging about 8 μm in diameter. The cell surface was densely covered with microvilli. Each microvillus had a diameter of about 0.12 μm, while the length varied from 0.3–1 μm (fig. 2). There were about 11 microvilli per μm^2 (table 1). At 15 min after microwave irradiation, the tumor cells appeared to form larger aggregates (fig. 3). Each microvillus measured 0.15 μm in diameter and about 1.2 μm in length. The number of microvilli increased to 24 per μm^2 (table 1). Cell aggregates were formed by extensive interdigitation of microvilli (fig. 4). After 25 min of microwave irradiation, the increase in the number of microvilli was more marked. More microvilli were found at cell junctions (fig. 5). Each microvillus measured about 1.3 μm in length and there were 39 microvilli per μm^2 (table 1).

After incubation of an EAT cell suspension in water bath at 41 °C, the cells appeared to form cytoplasmic blebs separate from the microvilli. Most of the blebs were globular or spherical in shape (figs. 6, 7). After incubation of tumor cells in a water bath for the same period at 46 °C, these blebs enlarged and some seemed to separate from the cells. In addition, the microvilli appeared to decrease in number (fig. 8).

Discussion. In this study, the ultrastructural changes of the surface morphology of EAT cells treated in vitro with microwave irradiation and heat are compared. The surface morphology revealed marked and severel changes during such treatments. Microwave irradiation caused an increase in both length and number of surface microvilli. Table 2 shows that the increases in the number and length of surface microvilli in EA cells after microwave irradiation are significant as compared to their control, untreated counterparts. A significant increase in the number of microvilli in EA cells treated with microwaves for 25 min is also noted as compared to those treated for 15 min. However, there is no significant increase in length of microvilli when the same groups of cells are compared. Tumor aggregates commonly occurred and cells were held together by means of extensive interdigitations of surface microvilli. On the

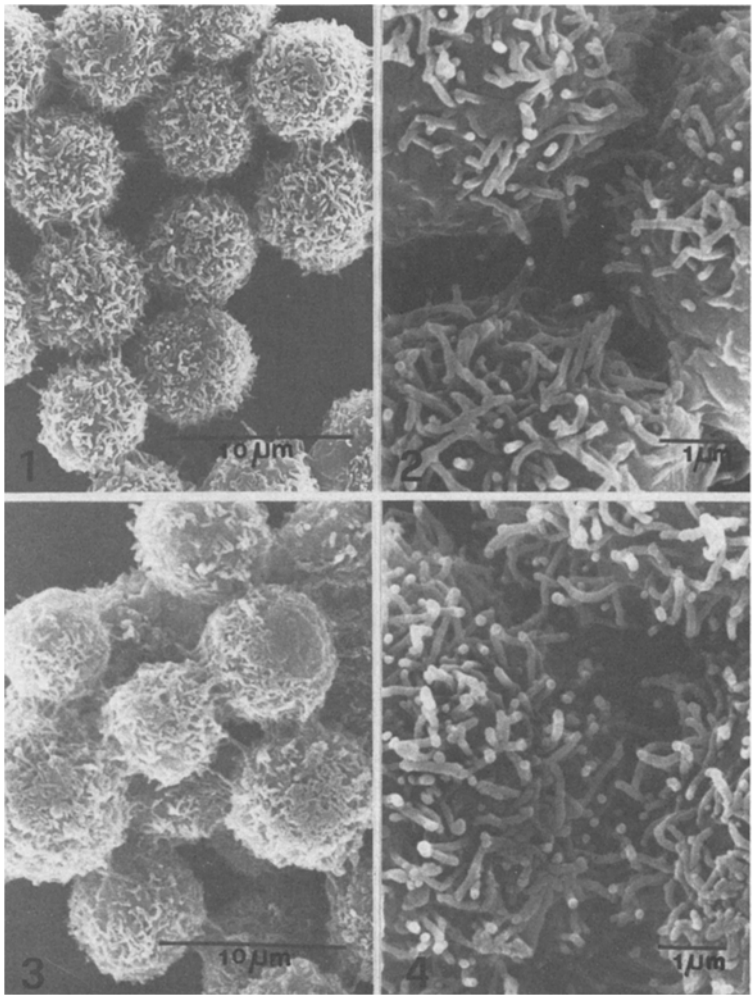

Figure 1. Survey micrograph showing untreated EAT cells in small aggregates.

Figure 2. Higher power view of areas of contact in untreated EAT cells.

Figure 3. Survey micrograph showing a small EAT aggregate 15 min after microwave irradiation.

Figure 4. High power view of part of an area similar to figure 3 showing increase of microvilli at intercellular junctions.

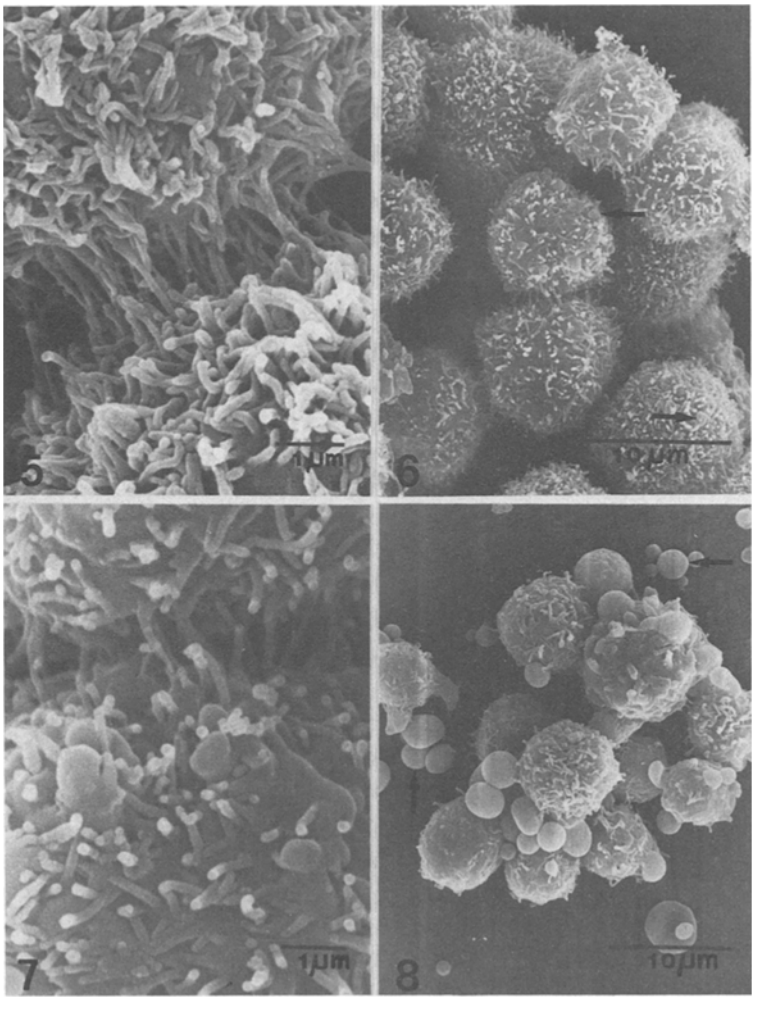

Figure 5. Part of 2 EAT cells showing extensive interdigitation of microvilli 25 min after microwave irradiation.

Figure 6. An aggregate of EAT cells 30 min after incubation in a water bath at 41 °C. Note the presence of cytoplasmic blebs (arrows) on the cell surface.

Figure 7. Higher power view of part of figure 6 showing cytoplasmic blebs amongst microvilli.

Figure 8. An aggregate of EAT cells 30 min after incubation in a water bath at 46 °C. Cytoplasmic blebs are present on the cell surface but some have detached (arrows).

other hand, heat hyperthermia caused a decrease in the number of surface microvilli but an increase in cytoplasmic blebs. Cavaliere et al.[18] reported that temperatures of 42–44 °C caused irreversible damage to Novikoff hepatoma cells but not to normal or regenerating rat liver cells nor to minimal deviation hepatoma cells 5123. Giovanella et al.[10] investigated thermal effects on normal (embryonic) and neoplastic mesenchymal cells and found that 95% of all cultures of tumor-derived and tumor-producing cells died after 21 hours at 42 °C, whereas only 43% of all cultured normal and non-tumor producing cells died under similar conditions. One of the major factors in cell death at $\geqslant 42$ °C is the irreversible damage to cell respiration[19,20]. Overgaard[9] believes that a primary, lysosomally conditioned, selective destruction of the malignant cells occurs and that this reaction is intensified by a high acidity in the tumor milieu. In our study, cell destruction was minimal and the viability of the EAT cells was tested by inoculation of microwave irradiated tumor cells into the peritoneal cavity of healthy mice. All 5 mice receiving these cells developed tumors. This proved that the intensity of microwave irradiation used is sub-lethal and the surface changes may be reversible.

The CHO^3 cell mutants deficient in cholesterol have more fluid membranes[21,22] and a positive correlation between cholesterol concentrations and cellular resistance to hyperthermia[23]. The marked increase of microvilli after 25 min of microwave irradiation at a sub-lethal level is probably caused by a further change in the membrane fluidity of EAT cells. Our preliminary results seem to indicate that the primary change in EAT cells in vitro under a sub-lethal level of microwave irradiation is at the cell membrane level, in contrast to the observations reported by other investigators. The differences in surface morphology after microwave irradiation and heat hyperthermia seem to indicate that the mechanisms of action of these procedures on EAT cells are not similar.

Enhanced dissemination of the Yoshida sarcoma in rats has been reported[3–5] as a result of local heating at temperatures inadequate for complete tumor destruction. Walker et al.[26] found the rate of proven metastasis following local curative hyperthermia to be more than double the most generous estimate of the metastasis rate following local curative radiation. Chew et al.[27,28] reported that the initial adhesion of EAT cells to substratum and mesothelial cells was accomplished by surface microvilli. Thus an increase in microvilli and cell aggregations after microwave irradation tends to favor metastasis and may account for the above phenomenon.

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Bombesin-like immunoreactivity in the pancreas of man and other mammalian species[1]

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Summary. Bombesin-like immunoreactivity has been measured in pancreatic tissues of man (12.4 ± 1.2 pmol/g), pig (15.8 ± 3.2), calf (4.3 ± 0.9), rat (8.5 ± 1.2) and guinea-pig (2.8 ± 0.6) by a specific radioimmunoassay. Gel filtration of the pancreatic extracts revealed 2 major immunoreactive peaks: the earlier peak was eluted in the position of porcine gastrin-releasing peptide, and the later peak was eluted just after the amphibian bombesin standard. Immunocytochemistry demonstrated the presence of bombesin-like immunoreactivity in nerves in the rat pancreas, particularly in the exocrine pancreas, and occasionally in the peri-insular spaces. Isolated rat pancreatic islets were found to contain small quantities of bombesin-like immunoreactivity (0.037 ± 0.003 fmol/islet) suggesting that mammalian bombesin-like peptides may be invovled in the regulation of endocrine as well as exocrine pancreatic secretion.

Mammalian bombesin-like or gastrin-releasing peptide (GRP)-like immunoreactivities have previously been shown by immunocytochemistry to be present in the intrinsic nerves of the gut[3] including the intrapancreatic ganglia of the pig[4], where the peptides with these immunoreactivities may act as neurotransmitters or neuromodulators[3,4]. Infusion studies with amphibian bombesin and GRP, the larger molecular form of bombesin-like immunoreactivity (BLI) isolated from the pig, have shown that these peptides have a wide spectrum of effects on pancreatic endocrine and exocrine functions including stimulation of glucagon, insulin and pancreatic polypeptide release[5,6] and pancreatic enzyme secretion[7,8,9]. In view of the possible role of the bombesin-like neuropeptides in the regulation of pancreatic endocrine and exocrine secretion we decided to quantify these peptides in the pancreas of human and other mammalian species by specific radioimmunoassay and to characterize their molecular forms by gel-permeation chromatography. In addition, isolated rat pancreatic islets were assayed for BLI and the rat pancreas was examined by immunocytochemistry to establish the precise localisation of this immunoreactivity.

Materials and methods. Tissues. Eight histologically normal fresh specimens of human pancreatic tissue were obtained immediately post mortem, or during therapeutic splenectomy or laparatomy in patients suspected of having endocrine tumors. Pancreatic tissues from 4 pedigree Jersey calves (aged 27–38 days) and 4 piglets (aged 6–8 weeks) were removed during anesthesia. Pancreatic tissues from 8 Wistar rats (250–350 g) and 6 Duncan Hartley guinea-pigs (350 g) were removed immediately after death by stunning and decapitation. In addition, pancreatic islets were isolated from 7 groups of 4 rats by a modification of the Lacy-Kostianovsky method[10] and extracted by boiling in 1 ml of 0.5 M acetic acid.

Extraction. Weighed portions of the tissues were immediately minced and extracted in 0.5 M acetic acid (10 ml/g) at 100 °C for 10 min, then frozen and stored at −20 °C until assay.

Radioimmunoassay. The tissue extracts were thawed, centrifuged, and duplicate aliquots of 10 µl and 1 µl of the supernatant were assayed for BLI. Antiserum (BN103) was raised in a rabbit against a (lys[3])-bombesin analogue conjugated to bovine serum albumin with glutaraldehyde[11] and used at a final dilution of 1:640,000. This antiserum crossreacted 96% with synthetic porcine gastrin-releasing peptide (GRP), 0.2% with substance P, and had no crossreaction with other gastrointestinal and pancreatic peptides tested[11]. The radiolabel was ^{125}I-(Tyr[4])-bombesin prepared by the chloramine T method[11] and subsequently purified on a column of Sephadex G-25 (fine) eluted with 0.1 M formic acid containing 1% bovine serum albumin. The assay standards were prepared gravimetrically from synthetic porcine GRP. After 6 days incubation at 4 °C, free label was separated from antibody bound label by adding 2 mg of

Pancreatic content of bombesin-like immunoreactivity in different mammalian species

Species	n	pmol/g wet weight of tissue
Rat	8	8.5 ± 1.2
Guinea-pig	6	2.8 ± 0.6
Calf	4	4.3 ± 0.9
Pig	4	15.8 ± 3.2
Human	8	12.4 ± 1.2
Rat (isolated islet preparations)	7	0.037 ± 0.003 fmol/islet