treated for an additional half hour with colcemid (0.03 µg/ml; Gibco)[9]. The rest of the technique for harvesting of the cells is standard. The CBG technique was performed on prometaphases and the cells were photographed on Kodak technical Pan film No. 2415 using a Zeiss Photomicroscope II.

Results and discussion. Differentiation of secondary constriction regions *h* by the CBG-technique in chromosomes 1, 9 and 16 is shown in the figure. When chromosomes were C-banded at metaphase such differentiation was never seen. However, at prometaphase or prophase the *h* regions differentiate into light and dark bands. These observations suggest that the *h* regions are composed of euchromatin (light staining areas) as well as heterochromatin (dark staining areas) which suggests the presence of different satellite DNAs in these regions. Some understanding about the properties of the *h* regions has been obtained recently by the use of BrdU in conjunction with fluorescent dyes such as DAPI[10]. It is believed that the heterochromatin of the region is late replicating[10]. However, the present observations suggests that the whole region does not replicate at the same time as it differentiates into light and dark bands. Furthermore, the definition of constitutive heterochromatin in man by C-banding needs further investigation. Although a possible clinical significance of the heteromorphisms of *h* regions has been suggested, no serious attempt has been made to conduct the study in a systematic fashion. Perhaps the present approach might lead to some conclusion regarding variability of staining properties of the *h* region with respect to clinical consequences.

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Teratogenic effects of methylnitrosourea on pregnant mice before implantation

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Summary. Methylnitrosourea at a dose of 10 mg/kg is teratogenic when applied to pregnant mice on gestational days 2.5, 3.5 or 4.5, but has no such effects on gestational days 0.5 and 1.5.

In teratological studies, it has generally been considered that treatment of pregnant animals with teratogenic agents during their preimplantation period results either in embryonic death or intact live fetuses, but no specific malformations[1,2]. However, some agents such as X-rays[3,4] cyclophosphamide, nitrogen mustard, thalidomide[5], triparanol[6], leucine[7], actinomycin D[8], aminoacetonitril[9], and a synthetic analog of 3β-hydroxysteroid dehydrogenase[10] have been shown to yield malformed fetuses when they are given to pregnant animals before implantation. Napalkov and Alexandrov[11], and also Tamaki et al.[12], have briefly described a few fetuses malformed by application of methylnitrosourea (MNU) or ethylnitrosourea (ENU) to pregnant rats in the preimplantation period. The teratogenic effects of MNU treatment of pregnant mice during their gestational days before implantation are investigated in this study.

Materials and methods. Virgin *slc:* ICR mice were housed with males of the same strain overnight, and the presence of a vaginal plug following mating was considered as marking day 0 of gestation. MNU (Nakarai) was dissolved in distilled water just before injection, and applied i.p. to mice at a concentration of 10 mg/kg from 13.00 to 13.30 h on gestational days 0 (day 0.5), 1 (day 1.5), 2 (day 2.5), 3 (3.5) and 4 (day 4.5), or 5 mg/kg and 1 mg/kg on gestational days 2.5, 3.5 and 4.5. Each mouse was injected with 0.05 ml/g of MNU solution. Mice given 0.05 ml/g distilled water and untreated mice were used for the controls.

On the morning of gestational day 18, the animals were killed by over-anesthesia, and the number and position of live and dead fetuses were noted. Dead fetuses were subdivided into early deaths (fetuses completely resorbed) and late deaths (fetuses remain). Live fetuses were sexed, weighed, and examined for external malformations. For the statistical analysis, the litter rather than the fetus was taken as the experimental unit, according to the recommendation of Haseman and Hogan[13]. The fetal responses were presented as the average for each litter within each group in the accompanying table 1. Except for mean fetal body weight, the fetal responses in each group were examined using Willcoxon's rank sum test. Student's t-test was used for the analysis of mean fetal body weight.

Results and discussion. No significant differences were observed in the number of implantation sites between all the MNU-trated groups and the control groups (table 1), indicating that the preimplantation mouse embryos in the MNU-treated mothers can survive at least until implantation. Spielmann and Eibs[14] also reported that treatment of pregnant rats with cyclophosphamide during the preimplantation period did not influence the number of implantation sites. Iannaccone et al.[15] reported that the mouse blastocysts which had been treated in vitro with MNU could implant to the same degree as the untreated blastocysts after transferring into the uteri of pseudopregnant mice.

The mean fetal death rates were significantly increased in the groups treated with 10 mg/kg MNU on gestational days 0.5, 2.5, 3.5 or 4.5, and with 5 mg/kg MNU on gestational days 2.5, 3.5 or 4.5, but in the 1 mg/kg MNU-treated groups only that in the group treated on gestational day 4.5 was signi-

Table 1. Effects of exposure to methylnitrosourea (MNU) on mouse fetal development

Gestational day	Treatment	No. of dams	Mean No. (±SD) of implantations	Mean (±SD) fetal death rate (%) Early	Late	Total	Total No. live fetuses	Mean (±SD) fetal body weight (g)	Total No. malformed fetuses	Mean (±SD) malformation rate (%)
0.5	MNU 10 mg/kg	15	13.9 ± 1.6	15.1 ± 10.7[b,d]	3.3 ± 5.9	18.4 ± 9.6[a,d]	170	1.34 ± 0.10	0	0
	H_2O	10	13.2 ± 2.7	3.3 ± 4.4	3.9 ± 6.1	7.2 ± 6.1	123	1.32 ± 0.14	1	1.3 ± 3.8
1.5	MNU 10 mg/kg	15	14.3 ± 1.7	15.5 ± 14.2	2.1 ± 4.7	17.8 ± 13.9	179	1.28 ± 0.10[b]	0	0
	H_2O	10	13.7 ± 1.6	4.3 ± 3.6	2.9 ± 4.8	7.2 ± 7.3	127	1.33 ± 0.09	2	1.4 ± 2.8
2.5	MNU 10 mg/kg	15	13.9 ± 2.3	65.8 ± 14.4[a,c]	3.5 ± 4.9	69.4 ± 14.9[a,c]	64	1.11 ± 0.20[a,c]	11	25.8 ± 34.9[b,c]
	5 mg/kg	15	15.0 ± 1.2	30.7 ± 15.6[a,c]	4.0 ± 5.2	34.7 ± 19.1[a,c]	147	1.25 ± 0.12[b]	2	2.1 ± 6.3
	1 mg/kg	15	14.3 ± 2.2	10.0 ± 9.9	5.0 ± 6.7	14.5 ± 9.9	183	1.34 ± 0.09	1	0.6 ± 2.1
	H_2O	10	14.1 ± 0.8	8.7 ± 6.3	3.5 ± 3.5	12.2 ± 7.4	124	1.36 ± 0.10	0	0
3.5	MNU 10 mg/kg	15	13.1 ± 2.2	30.5 ± 15.1[a,c]	7.1 ± 8.9	37.6 ± 16.3[a,c]	122	1.06 ± 0.13[a,c]	20	16.0 ± 11.8[a,c]
	5 mg/kg	15	14.1 ± 2.1	15.9 ± 8.5[a,d]	8.1 ± 8.3	23.4 ± 8.3[a,c]	161	1.23 ± 0.07[a,c]	6	3.0 ± 5.3
	1 mg/kg	15	13.7 ± 2.7	13.6 ± 10.2[b]	4.6 ± 6.6	18.2 ± 11.6[a]	170	1.34 ± 0.12	0	0
	H_2O	10	14.5 ± 1.3	7.6 ± 6.3	2.8 ± 3.4	10.4 ± 6.3	130	1.36 ± 0.14	0	0
4.5	MNU 10 mg/kg	15	13.0 ± 2.2	16.9 ± 12.5[a,c]	10.1 ± 11.0[b,d]	26.8 ± 15.4[a,c]	141	1.04 ± 0.10[a,c]	22	17.1 ± 22.1[a,c]
	5 mg/kg	15	13.2 ± 1.5	11.9 ± 9.4	8.7 ± 9.0[b]	20.6 ± 13.2[a,d]	143	1.18 ± 0.10[a,c]	2	1.1 ± 2.8
	1 mg/kg	15	12.9 ± 2.3	16.7 ± 14.9[a,c]	3.9 ± 5.8	19.9 ± 15.6[a,c]	154	1.22 ± 0.14[a,c]	8	5.4 ± 8.9
	H_2O	10	13.1 ± 2.7	6.9 ± 6.1	2.6 ± 4.1	9.5 ± 6.5	119	1.37 ± 0.11	2	1.4 ± 4.3
Untreated control		20	14.0 ± 2.0	5.5 ± 5.6	1.9 ± 4.0	7.4 ± 7.0	261	1.35 ± 0.08	3	1.2 ± 5.0

[a] $p < 0.01$ compared with untreated control group; [b] $p < 0.05$ compared with untreated control group; [c] $p < 0.01$ compared with respective H_2O-treated group; [d] $p < 0.05$ compared with respective H_2O-treated group.

ficantly increased compared with the control groups (table 1). These higher rates of fetal death mostly involved more early fetal deaths, but the rate of late fetal death in the group treated with 10 mg/kg MNU on gestational day 4.5 was also significantly increased (table 1). In the 10 mg/kg MNU-treated groups, the peak mean fetal death rate was observed in the group treated on gestational day 2.5 (table 1). Gebhardt[16] also observed the same increased embryo-death of 2.5-day mouse embryos after treatment of pregnant mice with cyclophosphamide or X-ray (in that study, the plug day was called day 1 of pregnancy, so day 3 of pregnancy in Gebhardt's study corresponds to gestational day 2.5 in the present study).

The mean fetal body weights were significantly reduced in groups treated with 10 mg/kg MNU on gestational days 2.5, 3.5 or 4.5, with 5 mg/kg MNU on gestational days 3.5 or 4.5, and 1 mg/kg MNU on gestational day 4.5, as compared with the control groups (table 1). In the 10 mg/kg MNU-treated and 5 mg/kg MNU-treated groups, the later the gestational day of MNU treatment was, the more the mean fetal body weight tended to be reduced.

In the 10 mg/kg MNU-treated groups, the mean malformation rates were significantly increased in the groups treated on gestational days 2.5, 3.5 and 4.5, as compared with the control groups (table 1). The types of malformations observed were various, but exencephaly, cleft palate and abnormal tail were conspicuous in each group (table 2). No significant increases were observed in the mean malformation rates of all the 5 mg/kg MNU-treated groups (table 1), but some types of alformations which were never found in the control groups were noted (table 2). In the 1 mg/kg MNU-treated groups, only sporadic malformed fetuses with cleft palate were present (tables 1 and 2).

There might be various mechanisms causing congenital malformations resulting from treatment with teratogenic agents of pregnant animals on their gestational days before implantation. Some agents may injure certain maternal tissues which are necessary for the maintenance of normal embryonic development, and malformed fetuses may result from these damaged maternal functions. On the other hand, as in the case of a synthetic analog of 3β-hydroxysteroid dehydrogenase[10], certain chemical agents may at first bind to the maternal tissues, and then be gradually released into the maternal blood and act directly upon the embryos later in their organogenetic period. Both of these mechanisms, however, seem to be inap-

Table 2. Types and numbers of external malformations after exposure to methylnitrosourea (MNU)

Gestational day	MNU dose (mg/kg)	Total No. of live fetuses	Total No. of malformed fetuses	Exencephaly	Spina bifida	Open eyelid(s)	Microtia	Cleft palate	Umbilical hernia	Polydactyly	Oligodactyly	Abnormal tail	Multiple malformations*
2.5	10	64	11	4				7				4	
	5	147	2	1									1
	1	183	1					1					
3.5	10	122	20	4		5		8	2		1	4	
	5	161	6	3	1			1		3			
	1	170	0										
4.5	10	141	22	4	1			6		1	4	7	
	5	143	2	1	1		1			1			
	1	154	8	1				7					
All the H_2O-treated groups		611	5					5					
Untreated control		261	3					3					

* Exencephaly, spina bifida, open eyelids, umbilical hernia and oligodactyly.

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%.