A-10
ISHIKAWA, H., K. OMOE and A. ENDO, Department of Hygiene and Preventive Medicine, Yamagata University School of Medicine, Yamagata. "Catch-up" phenomenon of digit formation in mouse fetuses from delayed fertilization.

We previously observed that digit formation in mouse embryos from early morning mating progresses to the extent that it does not differ from that from overnight mating. If such a catch-up phenomenon exists throughout the whole stage of gestation, the time-spectrum of the critical period for organogenesis must shift accordingly. In this study, to examine whether the embryos from normal and delayed matings (three, six or nine hours delay) respond differently to some acute teratogens when they are treated at the same time-point from mating, 5 mg/kg of cytosine arabinoside (Ara-C) was given to pregnant mice intraperitoneally at 246, 249 or 252 hours after mating.

The results showed that the patterns of Ara-C-induced anomalies in embryos from delayed mating were of more advanced stages, when compared to the groups with the same time intervals from mating. In other words, oocytes fertilized up to nine hours after the presumed time of ovulation could grow similarly to those of normally fertilized oocytes at their time-schedule of development. This suggests that the "ovulation clock" may be governing the growth and development of oocytes rather than "fertilization clock".

A-11
OHMORI, H., T. KOBAYASHI and M. YASUDA, Department of Anatomy, Hiroshima University School of Medicine, Hiroshima. Development of mouse cerebellum following oral administration of phenytoin (PHT) in neonatal period.

Phenytoin (PHT) is a commonly prescribed anticonvulsant drug.

Chronic PHT administration to epileptic patients may cause cerebellar dysfunction and degeneration. PHT is weakly acidic and slightly soluble in water. Recently, it was reported that oral administration of oil suspension of PHT results in a significant increase of its bioavailability.

The purpose of this study is to examine neurotoxic effects of PHT on the development of the mouse cerebellum.

PHT suspended in sesame oil was administered by gavage to newborn Jcl:ICR mice with 50 mg/kg body weight once a day for the period of postnatal days 2-14. The mean particle size of PHT powder was 17 μm.

Oral administration of PHT in neonatal period reduced total brain weight, cerebellar weight, and size of the cerebellum (transverse width and longitudinal width) measured on postnatal day 56. In reflexologic tests, placing response and negative geotaxis were poorly developed. Pyknotic cells in the external granular layer significantly increased and were prominent in the vermis area of the cerebellum from 14-day-old mice treated with PHT.

These results indicate that oral administration of PHT in the neonatal period produces neurotoxic injuries in the developing cerebellum.

A-12
YAMAMOTO, Y., K. YAMAMOTO, Y. FUKUI and A. KURISHITA, Department of Legal Medicine, Faculty of Medicine, Kyoto University, Kyoto, and Department of Radiation Research, School of Medicine, Tohoku University, Sendai, Miyagi. Teratogenic effects of methamphetamine.

The spread of methamphetamine (MAMP) abuse is a serious problem especially in the case of pregnant women, because this drug may have harmful effects on the fetus. There are, however, few reports on this problem. We reported that MAMP has teratogenic effects on mice and that environmental factors such as caging conditions have a profound effect on the mortality rate. In the present study, the effect of housing conditions on the rate of malformations (gross and skeletal) were examined.

Preganat Jcl:ICR mice were caged individually or in groups of three. On day 8 of gestation, they were given an intraperitoneal injection of MAMP hydrochloride at a dose of 21 mg/kg. On day 18 of gestation, they were killed and fetuses were removed and examined for gross and skeletal malformations.

There was a significant difference in the malformation rate between the two groups. The animals kept in group showed a higher rate of malformation. Severe
malformations such as exencephaly were observed only in the grouped animals. The same was the case with costal anomalies (defect and fusion). It was confirmed that caging conditions have a profound effect on malformation rate as well as on mortality rate.

A-13
TAKANO, T., M. OHNO, T. YAMANO and M. SHIMADA, Department of Pediatrics, Shiga University of Medical Science, Ohtsu, Shiga. Congenital hydrocephalus in suckling hamsters caused by transplacental infection with parainfluenza virus type 3.

The possible occurrence of congenital hydrocephalus by viral infection of the mother was examined by inoculating pregnant hamsters intravenously with parainfluenza virus type 3 (PIV-3).

Female Syrian hamsters 9–12 weeks of age were mated with breeder males for 24 hours. Seven pregnant hamsters were inoculated with 0.5 ml of 1.0 × 10⁶ pfu/ml of PIV-3 into the left cervical vein on either day 11, 12, 14 or 15 of gestation. Brains of offspring born to the mother hamsters were examined 13 to 17 days after birth to confirm the possible occurrence and incidence of congenital hydrocephalus.

When PIV-3 was inoculated on the 11th or 12th day of gestation, all mothers aborted. The hamsters which had been inoculated on the 14th or 15th day of gestation gave birth on the day of term, i.e. the 16th day of gestation. Sixteen offspring of 28 alive at birth survived until 13 days of age. Three of these survivors showed a centro-occipital prominence of the skull. Macroscopic examination of the brains showed a bulged cerebral hemisphere. Histological examination on the brains of these hydrocephalic hamsters revealed marked ventricular dilatation with thinning of the cerebral cortex and aqueductal forking.

Results obtained in this experiment may indicate that a virus inoculated into the cervical vein can arrive in the fetuses after passing through the placenta and infect the central nervous system.

A-14

Cytomegalovirus (CMV) is the most significant infectious cause of congenital anomalies of the central nervous system. However, little is known about the timing of gestational stage and site of embryos. In the present study, we tried to infect mouse embryos with murine CMV (MCMV) in the early and middle gestational stages. For early embryos, we injected MCMV into blastocysts from BDF1 mice using a micromanipulator and returned them to the uteri of the pseudopregnant ICR mice. On day 11 of gestation, embryos were fixed in Bouin's solution, embedded in paraffin and subjected to serial sections for immunohistochemical detection of viral antigen-positive cells. We produced no infected embryo using this system.

Then, the conceptus on day 8.5 of gestation were injected with MCMV by the Jaenisch method (1985). Embryos from day 5.0 to 12.5 of gestation were analyzed immunohistochemically. Viral antigen positive cells were first observed in the placenta, then endothelial cells and mesodermal cells in the embryos. After day cells were first observed in the placenta, then endothelial cells and mesodermal cells in the embryos. After day 12.5 of gestation, viral antigen-positive cells were observed even in the neuroectoderm and eye balls.

A-15
SAITO, K., K. SUZUKI and S. MOTOYOSHI, Department of Veterinary Physiology, and Department of Veterinary Internal Medicine, Nippon Veterinary and Animal Science University, Tokyo. Lethal and teratogenic effects of radio frequency radiation on developing chick embryo.

Developing chick embryos were exposed to 428-MHz radio frequency (RF) radiation at a power density of 5.5 mW/cm² for more than 20 days. The calculated SAR using both values of the electric and magnetic fields ranged between 3.1 and 47.1 mW/kg. Temperature change in eggs of which inlet was replaced by 25% gelatin, was not detected by fluoroptic thermometer in the RF field. Ten eggs were incubated in each of seven experiments. The 1st and 4th experiments were sham operated for control.

The average hatchability was 84.2% and 38.0% in control and exposed eggs, respectively. In the RF exposed group, embryonic development was stopped within 10 days after the start of incubation, while the remainder (76.6%) died in the egg shell because of inability to hatch. Since the control eggs hatched on days 21 or 22, and the exposed ones on days 21–23, it was clear that prolongation of the incubation period occurred in the exposed groups. A functional abnormality consisting of creeping movement and inability to stand was found in 89% of the hatched embryos in the exposed group, but was not in the controls.

It was concluded that 428 MHz RF radiation to developing chick embryos induced embryolethal and/or teratogenic effects and delayed hatching. It was also demonstrated that these adverse biological effects were due to a direct action of the RF radiation and not its inherent thermal effect.

A-16
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