(P < 0.005, r = 0.726). In a second study, we gave 2% NaHCO₃ via drinking water to furosemide-treated females from Days 15 (one day prior to treatment with furosemide) to 20. There was a further increase in average maternal pH (7.52) and a marked increase in the incidence of fetal wavy ribs (87.6%) compared to the group treated with furosemide alone (27.6%). These results and the previous findings suggest that extracellular hypochloremic metabolic alkalosis is involved in the pathogenesis of furosemide-induced wavy ribs.

C-29
CHEN, S. F., K. MIYATA, S. OKU, T. TACHIKURA, T. YAMAZAKI and M. NAKAMURA, Department of Pediatrics, Faculty of Medicine, Kagoshima University, Kagoshima. Teratogenic effects of combined used of anti-kidney serum and E-64 in the rat.

The teratogenic effects of rabbit anti-rat-kidney serum (AKS) combined with E-64 (a thiol protease inhibitor) were examined. Wistar rats were injected with 10 or 20 mg/kg of E-64 on days 9 and 10 of gestation, and with a subteratogenic dose (1 mg/kg) of AKS on day 9 or 10. The most common malformations were hydronephrosis, hydrocephalus, ureteral dilatation, microphthalmia, and heart defects. When AKS was given on day 9 or 10, the incidence of malformations was about 7% (10 mg/kg group) and 24% (20 mg/kg group). This indicated that the types of malformation are similar to those produced by AKS and E-64, and that there is an additive effect between AKS and E-64. It supports the speculation that E-64 and AKS have a similar mechanism of action.

C-30

The present study was undertaken to determine whether the thymic remnant in the neck (TRN) could be changed by a teratogen. Pregnant Crj:CD rats were administered subcutaneously on day 10 of pregnancy with 6-aminonicotinamide at 4.0, 5.2, 6.4 and 8.0 mg/kg. On day 20 of pregnancy, the fetuses were removed from dams, weighed, and examined for external malformations. After fixation with 10% formalin, the fetuses were examined for internal and skeletal malformations.

TRN was classified into 3 types such as a persistent cord of thymic tissue type, an accessory thymic tissue type, and a mixed type.

A dose-related increase in the incidence of TRN was noted in the fetuses as well as that of 14th rib. While the incidence of persistent cord of thymic tissue type of TRN decreased, that of accessory thymic and mixed types increased in a dose related manner. In the 6.4 and 8.0 mg/kg groups, a discontinuous type of TRN was also observed that did not occur spontaneously in the Crj:CD rats. These results indicated that TRN could be regarded as indicator of teratogenic potency of a drug. However, it is necessary to conduct further studies to clarify the meaning of various types of TRN.

C-31
MINO, Y., H. MIZUSAWA and K. SHIOTA, Congenital Anomaly Research Center, Faculty of Medicine, Kyoto University, Kyoto, and Merrell Dow Research Institute, Merrell Dow Pharmaceuticals Co., Ltd., Hirakata, Osaka. Effects of anticonvulsant drugs on fetal mouse palates cultured in vitro.

Palates of day 12.5 mouse fetuses were cultured in a chemically-defined serum less medium and the effects of the anticonvulsant drugs on cultured palates were studied. Explanted palates were treated for 72 hr in vitro with 50-200 μg/ml diphenylhydantoin (DPH), 200-800 μg/ml sodium phenobarbital (PB), 12.5-400 μg/ml sodium valproate (VPA) or 3-100 μg/ml diazepam (DAZ). After 72 hr culture, the secondary palate of control explants closed similarly to in vivo. The fusion of palatal shelves was inhibited dose-dependently with DPH, VPA and DAZ. PB caused no significant inhibitory effects on palatal closure at concentrations up to 800 μg/ml. The in vitro toxic effects of anticonvulsants tested appeared to correlate with the relative in vivo teratogenic potential of the drugs. This palate culture system may be useful for screening of teratogenic agents.

C-32
MATSUMOTO, N., K. TOYONAGA and S. IIJIMA, Department of Hygiene, The Jikei University School of Medicine, Tokyo, and Department of Health Sciences, Yamanashi Medical College, Yamanashi. Developmental effects of nickel chloride on early mouse embryos in vitro.

Female mice (ten to twelve weeks old) were induced to superovulate and were mated overnight with males. The presence of a vaginal plug the following morning indicated day one of gestation. On day 4 of gestation, blastocysts were flushed from the uteri. They were transferred to eight-chamber culture slides containing modified Eagle's basal medium with various concentrations of NiCl₂ and were incubated six days. To determine the developmental effects of NiCl₂, development to the following end points was scored: formation of a trophoblast outgrowth, growth of the inner cell mass (ICM), and differentiation of the ICM into two layers.

In more than 90% of the untreated embryos the ICM grew on the monolayer, and in approximately 50% the ICM differentiated into two-layered structure. When blastocysts were
cultured for six days in NiCl₂, effects on forming trophoblast outgrowth and two-layer ICMs were observed dose-dependently at increasing NiCl₂ concentrations. Neither formation of trophoblast outgrowth nor differentiation of the ICMs into two layers in treated embryos were significantly different from control up to 0.6 μg/ml. Over 1.6 μg/ml of NiCl₂, significant differences were seen between treated and untreated embryos in these end points. (Supported by a Grant-in Aid No.63304038 from the Ministry of Education, Science and Culture of Japan.)

C-33

TSUTSUI, Y., A. KASHIMAI, N. KAWAMURA and M. NAGAHAMA, Department of Morphology, Institute for Developmental Research, Aichi Prefectural Colony, Kasugai, Aichi. Brain abnormalities of the neonatal mouse after intraventricular injection with murine cytomegalovirus in the late stage of gestation.

Brain abnormalities after birth are induced by intrauterine infection with cytomegalovirus (CMV). We previously reported a model system for brain abnormality induced by direct injection of murine CMV (MCMV) into the cerebral ventricles of mouse embryos on day 15 of gestation. Brains of offspring in the early neonatal period, until 14 days after birth, showed that some neurons in the cerebral cortex and hippocampus have special susceptibility to MCMV infection, resulting in atrrophy of these regions.

In the present study, brains of neonates 21 to 28 days after birth, which were injected with MCMV (5X10² to 1X10⁶ pfu) on day 15 of gestation, showed large cystic lesions extending to about 20% of the MCMV-injected offspring. The inner surface of these cystic lesions was covered with thin epithelial cells. Perivascular cuffs were often present near the cysts and there were few viral antigen-positive cells on the cyst walls. These findings suggest that cystic lesions may be formed due to regional anoxia caused by viral susceptibility to vascular endothelium. This system may provide an experimental model for periventricular cysts or porencephaly in humans.

C-34

TAKANO, T., Y. KAMIYA, M. OHNO, T. YAMANO and M. SHIMADA, Department of Pediatrics, Shiga University of Medical Science, Otsu, Shiga. Experimental myxovirus-induced hydrocephalus: Determination of critical period of development of hydrocephalus after intracerebral mumps virus inoculation.

This study was undertaken to determine the critical period for hydrocephalus.

Syrian hamsters, 2, 10, 30 and 50 days of age, were given a single intracerebral inoculation containing 2 TCD₅₀ of mumps virus. Animals were sacrificed on various days after inoculation, and the development of hydrocephalus was histologically examined. Immunohistochemical studies were then carried out to examine reactive changes of the glial cells using anti-gial fibrillary acid protein (GFAP) antibody. Localization of mumps virus antigen was also identified using hyperimmune serum to mumps virus.

Hydrocephalus developed not only in suckling hamsters inoculated 2 and 10 days after birth, but also in young adult hamsters inoculated 30 and 50 days of age. In all hydrocephalic animals, focal denuding of the ependymal cell was observed. However, aqueductal stenosis was observed only in suckling hamsters accompanied by marked periaqueductal proliferation of GFAP positive cells, and subsequently progressive and fatal hydrocephalus developed. The distribution of mumps virus antigen was mostly localized in the ependymal cell and choroid plexus. In hamsters inoculated 2 days after birth, however, mumps virus antigen was observed even on neurons of the cerebral cortex, hippocampus, midbrain and cerebellum.

These results suggest that in animals inoculated with mumps virus earlier in life, hydrocephalus becomes severer with aqueductal stenosis, though there was no apparent critical period of development of hydrocephalus.

C-35

EDWARDS, M.J., J. CAWDELL-SMITH, M.S.R. SMITH and J. UFFOLD, Faculty of Veterinary Science, University of Sydney, and Faculty of Medicine, University of N.S.W., Australia. Defects of the neural tube and head induced in early guinea-pig embryos by hyperthermia.

Hyperthermia between days 16 and 32 of pregnancy in guinea-pigs causes a number of serious developmental defects, but few defective newborn are seen after heat at earlier stages. The neural tube closes at day 13 of pregnancy. Heat at this stage commonly causes abortion at 30–35 days and although some fetuses survive to birth no newborn have had neural tube defects, suggesting that embryos with these defects were eliminated. To test this hypothesis, female guinea-pigs were exposed at 9 am and 3 pm on days 11, 12, 13 or 14 of pregnancy for 1 hr to 43±0.5°C, which elevated core temperatures by 3.5–4°C. They were killed on day 25 and the embryos examined. The results are summarized in Table 1.

Day No. Preg- No. with Total Abnormal Embryos

| Day | Preg | No. | Total | Abnormal Embryos | Abnormal embryos | Resorb- Mal- | Mal- Embryo(%) | Ing(%) formed(%) |
|-----|------|-----|-------|------------------|------------------|------------|-------------|-----------------|-----------------|
| 11  | 11   | 0   | 28    | 4(14)           | 0                | 0          |             |                 |
| 12  | 10   | 2   | 32    | 1(3)            | 4(13)            | 18(17)     | 27(14)      |                 |
| 13  | 32   | 141 | 106   | 8(13)           | 14(23)           | 8(7)       |             |                 |
| 14  | 21   | 62  | 62    | 8(13)           | 14(23)           | 8(7)       |             |                 |
| 35  | 0    | 118 | 21(17)|                 |                  |            |             |                 |

The results are summarized in Table 2.

Kyphosis- Microph- Neuronal Micro- Other Total scoliosis thalasia tube cephaly

Day