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higher in hotbred serum than in coldbred one.

When the amount of lipid peroxides in neonate serum was compared with that of adult one, it was higher in the neonate serum regardless of the difference in breed, indicating that the oxidative stress was stronger in neonate than in adult. However, it was hard to explain this phenomenon by the difference in the amount of

SOD and the  $O_2^{\cdot-}$ -scavenging activity, because no difference was observed between their sera.

These results showing the age-dependent differences in the  $O_2^{\cdot-}$ -scavenging activity and the amounts of antioxidative substances between hotbred and coldbred horses will provide useful information for susceptibility of horses to exercise-induced oxidative stress.

### Effects of hypoxia on p53-dependent apoptosis and the arrest of cell-cycle progression in X-irradiated human EB-B cells

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Ionizing radiation induces less efficiently cell death under the hypoxic conditions than under the aerobic condition. Therefore, the distribution of hypoxic cells in the tumor is thought to be critical against a cure rate of radiation therapy. The killing effect of ionizing radiation on cultured mammalian cells was usually judged by the loss of clonogenic activity. Recent studies proved that two types of cell death, necrosis and apoptosis, were main components of the loss of clonogenic activity. The present study was carried out to examine how the hypoxia influences the ionizing irradiation-induced apoptosis in cultured mammalian cells with the aid of a specially designed gas-exchangeable chamber. Human B cells transformed by Epstein-Barr virus (EB-B cells) were employed and exposed to X-rays with 0~24 Gy under the aerobic and hypoxic conditions. Qualitative and quantitative measurements of apoptotic cells were made by the flowcytometric analysis of propidium iodide-stained cells and showed that apoptotic cell death was less induced in hypoxic cells than in aerobic cells. Observation of ladder-like cleavage of DNA, which is

characteristic of apoptosis, in cells irradiated under both conditions further confirmed this phenomenon. Flowcytometric analysis also showed another aspect of X-irradiated cells that the arrest of cell cycle progression at the G1/S and G2/M borders due to X irradiation was weaker in hypoxic cells than in aerobic cells.

To explain these observations, the accumulation of p53 protein and the subsequent decrease in the activity of cell-cycle relating protein kinase cdk2 (cyclin-dependent kinase), which is regulated by the p53 protein, were measured by Western blot analysis and the immunoprecipitation/kinase assay methods, respectively, in X-irradiated aerobic and hypoxic cells. The results showed that X irradiation remarkably induced the accumulation of p53 protein followed by the decrease in the cdk2 activity in aerobic cells, but induced to a less extent in hypoxic cells. Examination of X-irradiated cells by pulse-field gel electrophoresis showed that DNA double-strand breaks were more induced in aerobic cells than in hypoxic cells, suggesting that the difference in the accu-

mulation of p53 protein between aerobic and hypoxic cells was attributable to that in the amount of DNA double-strand breaks between them.

In hypoxic cells, it was concluded that X irradiation induced less amount of DNA double-

strand breaks which resulted in the less accumulation of p53 protein followed by the less decrease in the cdk2 activity. The decrease in the cdk2 activity to a less extent yet enabled cells to precede to the next cell cycle, as reflected by the less induction of apoptotic cell death.

Population characteristics of the sea otter (*Enhydra lutris*)  
on Cape Lopatka, Kamachatka Peninsula

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Morphometric measurements and tooth annuli counts from 593 skulls were used to determine sex and age characteristics of the Asian sea otter (*Enhydra lutris*) on Cape Lopatka, Kamchatka Peninsula, Russia. Measurements were taken on sea otter skulls from natural death recovered between 1983 and 1989 (n=551) and during January, 1997 (n=42). Five known aged skulls from aquariums were used as reference for age determination.

1) In addition to determining ages from cementum annuli, season of birth and season of death were determined from the condition of the innermost and outermost annuli respectively.

2) A reliable method of sex determination of pups was developed using morphometric characteristics of known sex skulls as reference.

3) An analysis of skull and canine tooth measurements across ages revealed continuing growth and development of the rostrum and coronoid process of the mandible, perhaps re-

flecting recent adaptations of this species to a marine environment.

4) Based upon a life table constructed from estimated age and sex, it appears that the waters around the Lopatka peninsula are inhabited primarily by young males.

Although the Cape Lopatka region provides ample food resources, strong winds and other environmental factors may make it a less favorable habitat than the more protected areas of this population's distribution. This may explain the preponderance of young, presumably dispersing males along the peninsula. A similar dynamic may be occurring among recently expanding populations near the southern Kuril islands and eastern Hokkaido. Improved research methods and continued studies of the Kamchatka, Kuril, and Hokkaido populations will help clarify the life history characteristics and population dynamics of the Asian sea otter, thus contributing to its conservation and management.