

ABSTRACTS OF PAPERS PRESENTED AT THE
SYMPOSIUM ON BIOLOGY OF THE MEDAKA
NAGOYA, DECEMBER 4, 1982

DEVELOPMENTAL BIOLOGY OF THE MEDAKA EGG

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The medaka egg was first introduced by Amemiya (1928) as a suitable subject for research and teaching in the field of developmental biology. Using the medaka embryo, Yamamoto (1931-) observed the rhythmical movement of the embryo at the beginning of development and suggested its role in yolk utilization. Later, he made extensive studies on fertilization of this fish and proposed a "wave of fertilization" hypothesis (1944-). In the same laboratory, the hatching enzyme gland of the medaka embryo was discovered by Ishida (1944) and the properties of the hatching enzyme were reported. Detailed mechanism of hatching was studied by Yamagami (1970-) from both biochemical and morphological viewpoints. Normal stages of medaka development were described by Matsui (1949) and have since been accepted by many researchers working with this fish.

As to the energy metabolism of the medaka egg, measurement of oxygen consumption by a single egg was attempted by Nakano (1953) using a Cartesian diver respirometer, which was followed by studies on respiratory enzymes of developing embryos (Hishida and Nakano, 1954). Differentiation of the isozyme patterns of four dehydrogenases was analyzed by Nakano and Whiteley (1965), resulting in the first demonstration of the retinal isozyme of lactate dehydrogenase in fish. Later, Philipp and Whitt (1977) made detailed studies on the gene expression of lactate dehydrogenase in the medaka embryo. Phosphorous metabolism in the medaka egg was studied

by Yamagami (1960-) and Ohi (1961). Tsusaka and Nakano (1965) measured the turnover rate of phosphorus in oocytes using ^{32}P . Patterns of protein synthesis during oogenesis and early development were studied by Monroy et al. (1961) and Nakano and M. Ishida-Yamamoto (1972). Amino acid components of the medaka egg were also reported by Nakano and S. Yamamoto (1972).

Changes in membrane potential at fertilization were measured by Maeno et al. (1956), Hori (1958) and Ito (1960-). Recently, Nuccitelli (1980) found that fertilization occurs at wide ranges of membrane potentials (-80 to +48 mV) and suggested that there is no electrical block to polyspermy in the medaka egg. The role of calcium at the time of fertilization was first pointed out by Yamamoto (1944). Later, Gilkey (1978) demonstrated a propagating calcium wave upon fertilization using aequorin-injected eggs.

Electron microscopical studies of oogenesis and early development of medaka were carried out by M. Yamamoto (1963-). Morphological changes in the structure of the cortical layer were studied by Iwamatsu (1965-), using light and electron microscopes. Besides these studies on developmental biology, there are numerous works in related fields, such as genetics, endocrinology and radiation biology. In the past, the medaka was mainly used in genetic studies, but in recent years it has become a favorite subject for studies in many fields of biology, especially that of developmental biology.

SOME PROBLEMS IN KEEPING FISH STOCKS

T. Tomita

Laboratory of Freshwater Fish Stocks, Nagoya University, Nagoya 464

Among the many problems in keeping fish stocks are the following :

1. Fish stocks should be kept in two or more laboratories. In our laboratory, about 70 mutants of medaka, *Oryzias latipes*, four types of gold fish, *Carassius auratus*, one type of silver crucian carp, *Carassius auratus longsdorfi*, and one type of common gambusia, *Gambusia affinis*, have been kept for many years. However, these fish stocks have been subject to great damage by natural disasters, such as flood caused by torrential rain on September 28, 1983. Unusually unseasonable temperatures and infectious diseases may deplete fish stocks. Although separate indoor

and outdoor fish stocks are kept at our laboratory, it is desirable to maintain stocks at other laboratories.

2. A large stock of the wild type of medaka should be maintained as the wild medaka has virtually disappeared in the suburbs of Nagoya and throughout Japan. Under such circumstances, it is necessary to maintain the wild population without changes in gene frequency. From a genetic resources point of view, more than 500 fish of the wild type should be kept in each stock. In our laboratory, the size of fish stocks is under 100 fish and, therefore, the gene frequency of fish may change in the following generations.

A CYTOGENETIC STUDY ON THE KARYOTYPE EVOLUTION OF THE INDIAN MEDAKA, *ORYZIAS MELASTIGMA*

H. Uwa¹, T. Iwamatsu² and O. P. Saxena³

¹Department of Biology, Faculty of Science, Shinshu University, Matsumoto 390, ²Department of Biology, Aichi University of Education, Kariya 448, and ³Department of Zoology, Multanial Modi College, Modinagar, India

Recent advances in cell-culture procedures and cytogenetic techniques have rendered it possible to provide detailed karyotypic data essential for determining the karyotype evolution and species differentiation in *Oryzias* fishes. In the present study, detailed karyotype studies are undertaken in the Indian medaka, *O. melastigma*, according to C-banding and silver staining procedures. Erythrocyte DNA content is also measured by cytophotometry.

The chromosome number is 48 in diploid consisting of 24 pairs of acrocentrics (NF=48). The first pair has secondary constrictions near the centromeric regions. The

silver staining procedure revealed the nucleolus-organizer regions (NORs) located on these secondary constrictions. Characteristic C-bands are also detected in the first and the second pairs. Estimated DNA-value in *O. melastigma* is 1.8 pg per nucleus. So, there is no significant difference in DNA-value from other *Oryzias* fishes.

Fishes of the genus *Oryzias* so far studied have been karyologically divided into three groups. It has been shown that 48 acrocentric chromosomes are recognized as a basic model complement of cyprinodontoid fishes including many other teleost groups. Thus, *O. melastigma* seems to be karyologically the

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basic species of the *Oryzias*.

EXPRESSIONS OF DOMINANT CHARACTERS OF *ORYZIAS LATIPES* IN THE HYBRID OF *O. LATIPES* AND *O. CEREBENSIS*

A. Iwata

Laboratory of Freshwater Fish Stocks, Nagoya University, Nagoya 464

In medaka, *Oryzias latipes*, two dominant genes have been found. The *Da* gene is incomplete dominant and the homozygotes have an anal fin in place of a dorsal fin, while the heterozygotes have a large dorsal fin which has an increased number of fin rays. The *Si* gene is complete dominant and causes a deficiency of semicircular iridocyte spots on the brain membrane. In the present study, the eggs of *O. latipes* were fertilized artificially with the sperm of *O. cerebensis*. In the

hybrid with the *Da*, the number of dorsal fin rays increased more than other hybrids. This fact indicates that the *Da* gene is expressed in the hybrid. The hybrid with *Si* had a semicircular spot of iridocyte on the brain membrane, and this fact indicates that the *Si* gene did not express itself. It may be concluded that the dominant characters of *O. latipes* are not always expressed in the hybrid of *O. latipes* and *O. cerebensis*.

PHARMACOLOGICAL ANALYSES OF THE MELANOPHORE RESPONSES OF DIFFERENT MUTANTS IN MEDAKA

K. Naruse, H. Tomita and E. Nakano

Department of Biology, Nagoya University, Nagoya 464

The effects of several chemicals (adrenalin, melatonin, caffeine, theophyllin and db-cAMP) on melanophores in scales isolated from the wild-type (*BR*) and some mutants (*cm*, *sm*, *dm*, and *dm-2*) of medaka, *Oryzias latipes*, were investigated. The melanophores of *sm* did not aggregate in the adrenalin-added isotonic balanced salt solution (BSS), while in melatonin-added BSS they concentrated. Further addition of either caffeine, theophyllin or db-cAMP into such melatonin-BSS made the concentrated melanophores disperse again. The melanophores of *cm* aggregated in BSS. With caffeine, theophyllin or db-cAMP in BSS, the melanophores of *cm* dispersed. The

melanophores of *dm* and *dm-2* were not responsive to any of the chemicals tested in the present experiment. These results suggest that the melanophores of *sm* may be deficient in adrenogenic receptors. The melanophores of *cm* were hypersensitive to adrenalin. This may be ascribed to the high affinity of adrenogenic receptors to adrenalin. For the negative responses seen in the melanophores of *dm* and *dm-2*, there are as yet no satisfactory explanations. However, from microscopic observations, the dendrites of the melanophores of these mutants were found to be slenderer than those of wild-type (*BR*).

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EFFECTS OF CYANOKETONE AND FOLLICLE CELLS ON GONADOTROPHIN- AND STEROID-INDUCED MATURATION *IN VITRO* OF *ORYZIAS LATIPES* OOCYTES

T. Iwamatsu

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In order to understand the mechanism of fish oocyte maturation by gonadotrophin, the effects of cyanoketone (CK) and follicle cells on the gonadotrophin- and steroid-induced maturation *in vitro* of full-grown oocytes were examined. In intact intrafollicular oocytes, breakdown of germinal vesicle (GVBD) occurred 7-8 h after initiation of incubation in the presence of 20β -OH progesterone, while the time (about 15 h) required for initiation of GVBD by pregnenolone was similar to that of gonadotrophin. It was, also, ob-

served immunocytochemically that a purified pregnenolone and gonadotrophin (PMS) stimulated *in vitro* production of progestins in the granulosa cells within 8 h after initiation of incubation, and that CK inhibited these stimulations.

These results indicate that maturation of *Oryzias latipes* oocytes is induced by MIS (progestins) metabolized from precursor steroids such as pregnenolone within follicle cells, especially granulosa cells, stimulated by gonadotrophin.

SOME PROPERTIES OF THE ISOLATED CORTEX OF THE MEDAKA EGG

M. J. Formacion, A. Hino and E. Nakano

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The cortex of unfertilized medaka egg was isolated in a medium ionically similar to the egg cytoplasm (200 mM ϵ -amino caproic acid, 5 mM NaCl, 2 mM MgCl₂, 10 mM EGTA or 1 mM CaCl₂ and 10 mM buffer). Cortical alveoli were released from the isolated cortex at the threshold level of 1 mM Ca²⁺ at pH 6.7. When phospholipase A₂ was added to the medium, the threshold level was lowered to 50 μ M Ca²⁺. ATP at 1 mM and 10 mM lowered it further to 1 μ M Ca²⁺. The response of the isolated cortex to phospholipase A₂ was not enhanced with 1 mM ATP but with 10 mM ATP, while the threshold level

was reduced to 1 M Ca²⁺.

Prominent modifications of the microvilli of the cortex were observed with the addition of ATP and phospholipase A₂ to the medium; e. g. enlargement, branching, elongation and formation of lobed tips. Small granules (diameter ranging from 0.25 - 0.60 μ m) were observed on the surface of the intact cortical alveoli. These granules disappeared during exocytosis of cortical alveoli. A schematic diagram of the process of cortical alveoli exocytosis was proposed and possible roles of ATP and phospholipase A₂ in the process was discussed.

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CLEAVAGE OF THE NEUTRAL RED-STAINED EGG OF MEDAKA

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Kojima (1959) showed that neutral red-stained granules may play important roles in cleavage in sea urchin eggs. In this report, whether neutral red (NR)-stained granules are present in the medaka egg and how cleavage proceeds in the NR-stained egg were examined. The eggs of medaka were stained with 0.001% NR in Ringer's solution before or after fertilization. Under observation with a scanning electron microscope (SEM), eggs were fixed with 3% glutaraldehyde in 0.1M phosphate buffer solution (pH 7.3) and 1% OsO₄. The staining of fertilized eggs was minimal and their development proceeded normally. Whereas in the staining of unfertilized eggs with NR, both sides of the cleavage plate were homogeneously stained during the

early cleavage stages and some NR-stained granules were recognized in the cytoplasm. In the normal morula, the blastoderm consisted of multilayers of blastomeres. In the NR-stained egg, the blastoderm became flat and consisted of blastomeres separated from each other in a monolayer, though cleavage proceeded. SEM observations showed that normal blastomeres became round just before cleavage and simultaneously many filopodia appeared along the contact region of two blastomeres. In the NR-stained egg, such processes were not detected.

These observations suggested that the formation of filopodia in the furrow region may play some role in configuration during cleavage.

RESPIRATORY METABOLISM DURING OOGENESIS IN MEDAKA, *ORYZIAS LATIPES*

F. Gomi, A. Hino and E. Nakano

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To analyze the mechanism of activation of fish eggs after fertilization, respiratory metabolism during oogenesis in medaka, *Oryzias latipes*, was studied. Adult fish were kept under controlled conditions of light (L : D=14 : 10) and water temperature (about 26°C). Ovaries were dissected and large oocytes were isolated. The rate of respiration of oocytes was measured by an oxygen electrode.

Fifteen hours before spawning (abbreviated as -15 h), the rate of respiration began to increase, reaching a plateau from -13 h to -9 h. From -9 h it again increased to a peak at -5 h. Germinal vesicle break down (GVBD)

occurred between -6 h and -5 h. After GVBD the rate of respiration decreased to about 1/10 of the maximum rate. Matured unfertilized eggs contained high levels of cyclic nucleotides.

The effect of 2,4-dinitrophenol, an uncoupling agent of oxidative phosphorylation, on the rate of respiration was also studied. The increased rate of respiration by 2,4-dinitrophenol showed a mirror-image-like change compared with that of normal respiration. When oocytes or adult females were centrifuged at 1,600 g for 5 min, germinal vesicles of oocytes moved into the yolk mass, and did not break down in this position. Centrifu-

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USE OF MEDAKA FOR MONITORING ENVIRONMENTAL CHEMICAL MUTAGENS

N. Egami and A. Shimada

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

We have reported that a dominant lethal test with male medaka is a useful tool for detecting environmental radiation. In order to apply this method to assay genetic toxicity of chemical mutagens in an aquatic environment, effects of some chemicals on the production of dominant lethality were examined in various inbred strains of the medaka. Males were treated with chemicals and mated with non-treated partners, and the hatchabilities of the eggs laid by each pair were counted daily. The dominant lethal mutation rate was calculated by the mean hatchability

as in our previous report.

Mitomycin C, methylmethansulfonate (M-MS) induced dominant lethals in early spermatids and spermatocytes as observed in mice. But the effects of ethylmethansulfonate (EMS), bleomycin, ethylurethane, methylurethane and phenylurethane were not clear. In particular, effects of EMS on fish were quite different from those of mammals. Experiments designed to study mechanisms of difference between mammals and fish in response to such chemicals are now in progress.

EXPERIMENTAL STUDIES ON THE BEHAVIOR OF RADIONUCLIDE ZINC-65 IN THE FRESHWATER ECOSYSTEM

K. Ijiri

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

Uptake and excretion of a radionuclide (^{65}Zn) were studied in freshwater worms (*Oligochaeta*) and fish (medaka, *Oryzias latipes*) to obtain basic information on the behavior of the heavy metal element in a laboratory aquatic ecosystem. Fish and a mass of worms were weighed and the ^{65}Zn content was measured by counting them in a small tube with nonradio-active water inserted into a gamma-scintillation counter. A rapid accumulation rate of ^{65}Zn by worms in water containing the isotope was observed.

Excretion of the isotope, however, was found to be a very slow process in this animal. Fish were then fed with such labeled worms for various periods, and their retention was followed with time in a nonradioactive water. The longest feeding period of 23 days with worms (2.0×10^5 pCi/g) showed the presence of a long-lived component in the excretion process in the fish. At 50 days half of the isotope content originally accumulated (4.0×10^4 pCi/fish body weight (g)) remained. A retention experiment after the uptake from

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Excretion of the isotope, however, was found to be a very slow process in this animal. Fish were then fed with such labeled worms for various periods, and their retention was followed with time in a nonradioactive water. The longest feeding period of 23 days with worms (2.0×10^5 pCi/g) showed the presence of a long-lived component in the excretion process in the fish. At 50 days half of the isotope content originally accumulated (4.0×10^4 pCi/fish body weight (g)) remained. A retention experiment after the uptake from

ged oocytes showed the maximum rate of respiration at -5 h, while oocytes at -3 h had a lower respiratory rate. These results sug-

gested that the inhibition of respiration is not dependent on GVBD, but may be due to qualitative changes in the cytoplasm.

USE OF MEDAKA FOR MONITORING ENVIRONMENTAL CHEMICAL MUTAGENS

N. Egami and A. Shimada

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

We have reported that a dominant lethal test with male medaka is a useful tool for detecting environmental radiation. In order to apply this method to assay genetic toxicity of chemical mutagens in an aquatic environment, effects of some chemicals on the production of dominant lethality were examined in various inbred strains of the medaka. Males were treated with chemicals and mated with non-treated partners, and the hatchabilities of the eggs laid by each pair were counted daily. The dominant lethal mutation rate was calculated by the mean hatchability

as in our previous report.

Mitomycin C, methylmethansulfonate (M-MS) induced dominant lethals in early spermatids and spermatocytes as observed in mice. But the effects of ethylmethansulfonate (EMS), bleomycin, ethylurethane, methylurethane and phenylurethane were not clear. In particular, effects of EMS on fish were quite different from those of mammals. Experiments designed to study mechanisms of difference between mammals and fish in response to such chemicals are now in progress.

EXPERIMENTAL STUDIES ON THE BEHAVIOR OF RADIONUCLIDE ZINC-65 IN THE FRESHWATER ECOSYSTEM

K. Ijiri

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

Uptake and excretion of a radionuclide (^{65}Zn) were studied in freshwater worms (Oligochaeta) and fish (medaka, *Oryzias latipes*) to obtain basic information on the behavior of the heavy metal element in a laboratory aquatic ecosystem. Fish and a mass of worms were weighed and the ^{65}Zn content was measured by counting them in a small tube with nonradio-active water inserted into a gamma-scintillation counter. A rapid accumulation rate of ^{65}Zn by worms in water containing the isotope was observed.

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labeled water was also conducted with fish. These experiments yielded parameters de-

scribing two or three compartments of zinc turnover.

MEDAKA AS EXPERIMENTAL ANIMAL FOR BASIC GERONTOLOGICAL RESEARCH

K. Kator

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

Recently, there has been a growing interest in the use of the fish as an experimental animal for studies on aging. Medaka (*Oryzias latipes*), which has been used as a good material in various fields of biology, is small in size and relatively short-lived (maximum life span : 5 years) and seems to be one of the most convenient fish for research on aging in the laboratory.

Several inbred strains of the fish were recently established by Y. Hyodo-Taguchi. One of the inbred strains, HB1-2, is more sensitive to ionizing radiation than the orange-red variety and its life span is estimated to be 1.5 years.

Recent data suggest that cells become more susceptible to radiation by reducing cellular superoxide dismutase (SOD) activity and that longer-lived species have a higher degree of protection against superoxide radicals. In the present experiment, the activity of SOD in the brain and liver of the orange-red variety and the inbred strain HB1-2 was investigated to elucidate the correlation between high sensitivity to radiation and short life span of the HB1-2 strain. The results showed that the total SOD activity was lower in both tissues of the HB1-2. The low activity of SOD may account for the high sensitivity to γ -radiation and short life span of the HB1-2 strain.

EFFECT OF LOW TEMPERATURE ON THE ALLOGRAFT REJECTION IN THE MEDAKA, *ORYZIAS LATIPES*

S. Kikuchi

Department of Biology, Faculty of Science, Chiba University, Chiba 260

In the medaka, *Oryzias latipes*, allograft rejection is greatly affected by the ambient environmental temperature. The 1st-set graft is rejected within 5-7 days at 25°C, however, the graft survives more than 80 days at 5-7°C. If the 2nd-set transplantation is performed between the 10th and 20th day, the graft is rejected within 2-3 days at 25°C. However, such a secondary response abruptly weakens.

The first experiment was performed as follows : 1) Three scales were transplanted from

donor to recipients. 2) The allograft rejection was assayed by the destruction of melanophores in the transplanted scales. 3) After the 1st-set transplantation the fish were maintained at 25°C and then were removed to 6°C on the 15th day after the 1st-set transplantation. 4) On the 50th day, the 2nd-set transplantation was performed and the fish were again transferred to 25°C. 5) Later destruction of melanophores was observed. Under these conditions, the 2nd-set grafts were re-

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jected within 3 days. The results indicated that the disappearance of the immunologic memory was suppressed by low temperature.

Another experiment was performed on specified days after the 1st-set transplanta-

tion, fish were removed to 6°C and 2nd-set transplantation was performed on the 15th day. From the results it was suggested that the memory had been formed before the 4th day.

COMPLEX POLYMORPHISM OF MUSCLE LDH IN THE MEDAKA, *ORYZIAS LATIPES*

M. Sakaizumi

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

The teleost fish has 3 lactate dehydrogenase loci. The isozyme A₄ is generally found in the muscle while the product of the locus B can be detected in many organs and tissues. The C locus in most teleost fish is specifically expressed in the eye retina. A₄ tetramer shows the least stability to heating, whereas the C₄ isozyme is most stable. These conditions are almost the same in the medaka, *Oryzias latipes*.

The muscle extract of wild populations of medaka demonstrated many different electrophoretic patterns. This is due mostly to the polymorphism of *Ldh-A^c* locus, which showed geographically specific distribution. Genetic

crosses revealed that this locus segregated for at least 5 alleles. *Ldh-A^c* was observed in all Japan except 2 areas. The most anodal allele, *Ldh-A^a*, was found along eastern coast of the Pacific and in Ryukyu Islands, and was usually a *Ldh-A^a/Ldh-A^c* heterozygote. The specimens from the eastern part of the Inland Sea district showed 3 major bands due to a more cathodal allele, *Ldh-A^d*. *Ldh-A^e*, the most cathodal allele to date, was found mainly in the San-in district. Specimens from this district had only 2 major bands. The hybrid individuals, *Ldh-A^c/Ldh-A^d* and *Ldh-A^c/Ldh-A^e*, were observed in the marginal zones of both districts mentioned.

IMMUNOREACTIVITY OF SOME TISSUES OF ADULT MEDAKA TO ANTICHORION ANTIBODY

K. Yamagami¹, T. Hamazaki¹ and I. Iuchi²

¹*Life Science Institute, Sophia University, Tokyo 102 and* ²*Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113*

In the course of an immunological study of choriogenesis in medaka using rabbit anti-chorion glycoprotein antisera (or IgG) as a probe, it was found that not only the chorion of oocytes in the ovary but also other tissues including the blood plasma, the endothelium of blood vessels, and the parenchymatous cells of liver in the egg-laying fish, were reactive to

the antibody. Some corresponding tissues in the adult male fish were unreactive. The egg yolk itself showed only slight immunoreactivity. Preliminary immunoelectrophoretic examinations revealed that the immunoreactive substance(s) in the liver, ovary and blood of the egg-laying fish showed an identical relative mobility (R_m)

jected within 3 days. The results indicated that the disappearance of the immunologic memory was suppressed by low temperature.

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(s) may be related to oogenesis, especially to choriogenesis, although it is a common knowledge that the egg chorion is synthesized in oocyte and follicle cells.

Analyses of the nature of this substance (s) are now being carried out.

RESPONSE OF PRIMORDIAL GERM CELLS OF *ORYZIAS LATIPES* TO γ -RAYS COMBINED WITH HEAT TREATMENT

Y. Shimada and N. Egami

Zoological Institute, Faculty of Science, University of Tokyo, Tokyo 113

The effects of γ -rays combined with heat treatment on primordial germ cells (PGCs) of the embryos of *Oryzias latipes* were examined and compared to those on intestine cells. It is well known in general that heat treatment potentiates radiation damage on cell proliferation. Certainly, the effect of radiation on intestine cell proliferation was enhanced in combination with heat treatment (41°C, 60 min) either before or after irradiation. On PGCs, however, the effect of radiation was protected (reduced) when combined with heat treatment administered

before irradiation at stage 28-29 of embryonic development (Matsui, 1949). A hypoxic condition before and during irradiation also functioned to protect PGCs from radiation damage at this stage. As both heat treatment and induction of a hypoxic condition are known as heat shock protein (hsp) inducers, we are now examining the effect of protein synthesis, inhibitors and other hsp-inducers on this response in order to elucidate whether or not this protective reaction among PGCs is related to hsp(s).

EFFECT OF X-IRRADIATION ON EMBRYONIC DEVELOPMENT OF THE INBRED STRAINS OF THE FISH, *ORYZIAS LATIPES*

Y. Hyodo-Taguchi

Division of Biology, National Institute of Radiological Sciences, Chiba 260

Embryos of two different inbred and outbred strains of the fish, *Oryzias latipes*, were irradiated with X-ray doses of 100 to 2000 rad at the morula stage. After X-irradiation, groups of about 40 embryos were incubated in water separately at 25°C in a petri dish. In the other groups, irradiated embryos were treated with 0.2 mg/ml caffeine for 21 h and

then rinsed with D. W. and placed in individual petri dishes to complete development and hatching. Survival rate at Stage 19 and hatchability were calculated on the basis of daily observation of the embryos.

From the dose-survival and dose-hatchability curves of each strain, a 50% survival dose (LD₅₀) at Stage 19 and a 50% hatch-

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ability dose HD_{50} were derived. LD_{50} at Stage 19 and HD_{50} in the inbred strain HO4C was 788 and 234 rad. In HB32C embryos, 788 rad of LD_{50} and 345 rad of HD_{50} were observed. LD_{50} (1140 rad) and HD_{50} (674 rad) in the embryos of the outbred were higher than those of the inbred strains. A

higher sensitivity among the inbred strain embryos of *Oryzias* to X-irradiation was clearly demonstrated. Caffeine treatment of X-irradiated embryos did not affect the survival rate at Stage 19, but a further decrease of hatchability was observed in the embryos of the inbred and outbred strains.