Effects of Radiation on the LDH-isoenzyme
and Chromosome of Developing Embryo and Embryonic Heart,
Special References to Abnormal Cardiogenesis

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ORGANOGENESIS is controlled by genetically
determined program, and gene activity is
easily influenced by the environmental factors
during the organogenetic period.

Radiation, one of the effective mutagen and
teratogen, can induce cardiovascular anomalies in
experimental animals.

Previous data demonstrated that a high inci-
dence of cardiovascular anomalies, especially of
transposition complexes, was induced by a single
dose of fast neutron irradiation to the 8 days
pregnant rat.

To analyse the related mechanism of ab-
normal cardiogenesis following fast neutron
irradiation, LDH-isoenzyme pattern and chromo-
some of embryos as the indicator of gene ab-
normalities were examined.

MATERIALS AND METHODS

For the production of the highest incidence of
cardiovascular anomalies, a single whole body
irradiation of 130 rad of 14.1 MeV fast neutron
was performed to Donryu strain pregnant rat
on 8 days of gestation. The embryo and heart
were removed under stereomicroscope. The
samples for LDH-isoenzyme analysis were
homogenized and centrifuged, and the super-
natant was electrophoresed in 5.5% acrylamide
gel at 2.5 mA. per gel. LDH was visualized in the
reaction mixtures described by Goldberg and
Cather. After fixation, relative intensity of
isoenzyme was measured in the microdensito-
meter.

For chromosome analysis, the direct method
was applied.

RESULTS

1. LDH-isoenzyme (Fig. 1–4)

Gradual increase of H-subunit during the
prenatal period was confirmed in normal
embryos and fetuses, and also in their hearts on
11–20 days of gestation. In the irradiated group,
changes of isoenzyme patterns showed some
individual difference, but in most of preparations,
the activity of LDH was markedly depressed
until 1–2 hours after irradiation. This indicated
an acute metabolic depression, and corresponded
to the period of mitotic arrest as shown in the
chromosome analysis. Following a short period of
metabolic depression abnormal LDH-isoen-
zyme patterns, especially unusually higher
amount of H-subunit such as LDH-1 or 2, were
observed in some cases during 2–12 hours.
However, most cases showed a lower activity of
LDH and decreased amount of H-subunit. At
this stage, immediate embryonic death following
irradiation might have occurred. During the
period of 6–12 hours after irradiation, the
average amount of LDH-H subunit decreased
compared with that of control. At 24 hours
after irradiation, the embryo restored the normal
level of H-subunit. During the period of 24–144
hours after irradiation, the tendency of delayed

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Fig. 1. Schematic drawing of LDH zymogram of whole embryo.
Short bands indicate that they were not found in every preparations.
Darker and wider bands indicate higher activity. Note retarded differentiation and abnormal occurrence of LDH-1 in irradiated group.

Fig. 2. Mean H-subunit amounts of LDH in whole embryo.
--- control --- irradiated
increase of H-subunit was noticed.

In the heart preparations, the minimal size of embryonic heart to be removed under stereomicroscope was that of 11 days of gestation (72 hours after irradiation) in the irradiated group. Mixed preparations of abnormal and normal hearts in the irradiated group showed the tendency of delayed increase of H-subunit during 72–120 hours. The abnormal heart ("pure" abnormal in Fig. 4) showed more retarded differentiation than that of mixed preparation. "Pure" abnormal heart in this stage may transform into transposition complexes in the term fetuses. At 144 hours after irradiation, the pattern of LDH-isoenzyme was considered to have returned to the normal range. During 48–96 hours after irradiation, embryonic death moderately increased (secondary death). Seven days after irradiation, neither abnormal pattern nor variant type of LDH-isoenzyme was noticed in spite of apparent formation of abnormal hearts. No abnormal LDH-isoenzyme pattern was observed thereafter. Consequently, the preparation of distinctive transposition complexes showed no abnormalities of LDH-isoenzymes.

2. Chromosome (Fig. 5)

After short period of mitotic arrest following irradiation, unstable type of aberrations appeared in a high incidence, and decreased gradually with the lapse of time. At 72 hours after irradiation, unstable type disappeared. On the other hand, stable type of aberrations appeared 4 hours after irradiation, and persisted until 120 hours, although the incidence was low. Aneuploid cells, especially hypodiploid cells increased shortly after irradiation, and then gradually decreased to the control level. Polyploid cells appeared during the period of 48–120 hours after irradiation, though the incidence was low.

DISCUSSION AND SUMMARY

Fig. 3. Schematic drawing of LDH-zymogram of embryonic heart. Note retarded differentiation in irradiated group.

Fig. 4. Mean H-subunit amounts of LDH in embryonic heart of control and irradiated groups. Note the most retarded differentiation in "pure" abnormal heart preparation.

- - - control  x — x irradiated, mixed
Genetic and environmental factors may be involved in the causal genesis of congenital heart disease. As to the environmental causes, various agents have been reported, but no agents were specific for individual anomaly.

In this study, fast neutron, one of ionizing radiation, was used for induction of congenital cardiovascular anomalies, and the transposition complexes were induced in about 60% of the term fetuses. This experiment was performed for the purpose of elucidating the teratogenic mechanism of cardiovascular system. The results obtained in this experiment agreed mostly with the data of radiation biology; During the first 1–2 hours after irradiation, initial metabolic depression was noticed, and was followed during 3–6 hours by direct radiation damage, such as cell death, decreased LDH activity, abnormal H-subunit amount and unstable type of chromosome aberration. Repair process was noticed during 6–24 hours, and recovery process during 24–144 hours after irradiation. These results were well correlated to the histological data previously reported.\(^2,4\)

However, these radio-biological explanations were not fully satisfactory for the induction mechanism of cardiovascular anomalies, because the embryo and its organs were of continuously differentiating organisms. Differentiation of the cell and tissue should be taken into consideration in addition to radio-biological explanation. Differentiation of organisms is controlled by differential gene activity. In normal embryo and embryonic heart, gradual increase of H-subunit of LDH indicates the advance of differentiation during the prenatal period. Accordingly, the lower amount of H-subunit observed in repair and recovery periods in the irradiated group indicated only retarded differentiation of embryo and embryonic heart. On the other hand, abnormal hearts were morphologically observed after 11 days of gestation in the irradiated group. As to the abnormal cardiogenesis, it should be considered as the abnormality of differentiation caused by differential cell death following irradiation in addition to retarded development of embryonic heart. Another phenomenon of abnormal differentiation was noticed in an abnormally high H-subunit of LDH observed during 3–24 hours of postirradiation period. This may also lead to erroneous differentiation of the heart. However, in the stage of biochemical recovery, distinctive cardiovascular anomalies were morphologically observed, and in the “pure” abnormal heart preparations most retarded differentiation was observed, but no
abnormal LDH isoenzyme pattern was noticed. The retarded and abnormal differentiation caused by differential cell death in the embryonic heart may play the greater part of induction mechanism of cardiovascular anomalies. Temporal abnormal activation of the gene shown by abnormally high H-subunit within 24 hours after irradiation in some cases may also play a role of the induction mechanism in a less extent. This explanation may be replaced by the loss and abnormality of genes which control the genetically determined organ differentiation of the heart.

The results of chromosome analysis almost completely agreed with the data of radiation biology.

From these results of radiation teratogenesis, the infant with congenital cardiovascular anomalies may be hardly associated with chromosome aberration, but in the induction mechanism the involvement of abnormal and retarded differentiation of gene activation caused by the cell death and temporal abnormal gene activations were suggested.

REFERENCES