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Developmental Genetics on the Lactate Dehydrogenase (LDH) Isozyme During Early Embryogenesis in Salmonids*

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Abstract

Lactate dehydrogenase (LDH) isozyme patterns were surveyed to obtain useful genetic markers in the developmental genetics among 5 species of salmonid fishes, masu salmon (*Oncorhynchus masou*), chum salmon (*O. keta*), coho salmon (*O. kisutch*), whitespotted char (*Salvelinus leucomaenis*) and dolly varden char (*S. malma*). There were electrophoretic differences in isozyme patterns of the LDH-B system between masu salmon and the other four species of salmonids. Developmental studies showed that only maternal LDH isozyme had appeared until the time of the tail bud stage characterized by the enclosure of blastopore and formation of the tail bud. Paternal isozyme subunit became detectable at this stage in the viable intergeneric hybrids, masu salmon ♀ × whitespotted char ♂, masu salmon ♀ × dolly varden char ♂ and the interspecific hybrid, masu salmon ♀ × coho salmon ♂. At about the same stage, changes in LDH isozyme pattern were also observed both in chum salmon and whitespotted char. The results obtained here suggest that the paternal gene activation on Ldh-B_1 and -B_2 loci may start from the tail bud stage in the embryogenesis of salmonids.

An effective approach in the developmental genetics is to hybridize animals each having phenotypically distinct isozymes. Analyses of the paternal gene activation by means of the zymogramic detection of paternal isozymic subunit during the embryogenesis of the resultant hybrid were carried out in some teleosts.[1-7] The present authors in the previous work[8] demonstrated that maternal isozyme pattern appeared during earlier stages of embryogenesis, and an additional isozyme containing paternal LDH-B^1 isozymic subunit also appeared from the tail but stage in the interspecific hybrid masu salmon ♀ × pink salmon ♂. Although these results suggested that the activation of paternal Ldh-B_1 gene occurred at the tail bud stage, we could not determine the activation time of paternal Ldh-B_2 gene in that interspecific hybrid experiment because the position of LDH-B_4^2 was overlapped between masu salmon and pink salmon.

As it has been proven in the present study that there is an allelic difference in Ldh-B_2 gene locus between masu salmon and two species of *Salvelinus*; whitespotted char, dolly varden char, we aimed to study further the activation time of the paternal Ldh-B_2 gene which originated from *Salvelinus* in the intergeneric hybrid masu salmon ♀ × whitespotted char ♂ and masu salmon ♀ × dolly varden char

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♂. In addition, another interspecific hybrid was also made between masu salmon ♀ and coho salmon ♂ to confirm the previous results[8] about the activation of Ldh-B_1 gene locus. The changes in LDH isozymic patterns in the normal embryogenesis of masu salmon, chum salmon and whitespotted char were also observed to collect evidence regarding the beginning of genomic functions during the embryogenesis.

Materials and Methods

Hybridization between masu salmon (*Oncorhyncus masou*) and whitespotted char (*Salvelinus leucomaenis*) were carried out by a single mating on October, 4, 1976 in Nanae Fish Culture Experimental Station, Faculty of Fisheries, Hokkaido University (Nanae is about 10 km north of Hakodate). The resultant combination, masu salmon ♀× whitespotted char ♂, whitespotted char ♀× masu salmon ♂, masu salmon ♀× masu salmon ♂ and whitespotted char ♀× whitespotted char ♂ were made at the same time. On October 14, 1976, single mating between masu salmon ♀ and dolly varden char (*S. malma*) ♀ and between masu salmon ♀ and coho salmon (*O. kisutch*) ♂ were also performed at the Nanae Fish Culture Experimental Station. All fish used for the hybridization were those reared in the ponds of the Nanae Experimental Station. After the insemination, eyes, hearts and livers were taken from each fish and frozen at –20°C for electrophoretic analyses to check parental lactate dehydrogenase (LDH) isozyme pattern. Fertilized eggs were transported to our laboratory of Faculty of Fisheries, Hokkaido University, Hakodate and incubated at an average temperature of 10.0 ±1.0°C. Artificial insemination of chum salmon (*Oncorhynchus keta*) was carried out in the Oshima branch of Hokkaido Salmon Hatchery at Yakumo, about 70 km north of Hakodate, on October 5, 1976. These salmon were caught from the Yurrap river at Yakumo. Fertilized eggs of chum salmon were transported to our laboratory in Hakodate and incubated at almost the same condition as those of the hybrid between masu salmon and whitespotted char and the others described above.

During the incubation period, fifteen to twenty embryos were collected in early stages before the eyed stage, and ten to fifteen embryos in later stages until hatching at a 3 day intervals and frozen at –20°C for electrophoretic analyses. In addition, some embryos were also taken and fixed with Bouin's solution for morphological observation to check the developmental stages. Starch gel electrophoresis for LDH was the same as described in the previous work.[8]

Results

LDH isozyme pattern

Lactate dehydronegase (LDH) isozyme patterns in eyes, hearts and livers in 5 speices of salmonids are demonstrated in Fig. 1. The terminology of LDH subunits and isozymes was basically accorded to that of Wright *et al.*[5] and superscript letters were applied in order to distinguish the origin of the LDH subunit and isozymes. As shown in Fig. 1, no species specific distinction was observed in the eye specific LDH-C system isozymes between *Oncorhynchus* (coho

salmon and chum salmon) and *Salvelinus* (whitespotted char and dolly varden char) both having 2 or 5 dominant bands. On the other hand, masu salmon presented a unique 5 banded LDH–C isozyme system in their eyes.

The LDH-B system was ubiquitously expressed in all organs studied and it resulted in five banded isozyme patterns in coho salmon, chum salmon, whitespotted char and dolly varden char. The isozyme patterns in the LDH-B system, however, appeared in a tissue specific manner. In the livers, strongly stained isozymic bands in LDH-B system were reversed between *Oncorhynchus* and *Salvelinus*. The livers of two species of *Oncorhynchus*, chum salmon and coho salmon, showed only one isozymic band at anodal side while two species of *Salvelinus*, whitespotted char and dolly varden char showed the predominant band at cathodal side (Fig. 1). Such a reversal of tissue specificity was also recognized in the hearts between *Oncorhynchus* and *Salvelinus* species (Fig. 1). These reversals indicate that the electrophoretic mobilities of LDH-B_4^1 and -B_4^2 were mutually reversed in these two genera.

Slight differences were detected in electrophoretic mobilities between anodal homotetramers $B_4^{2\circ}$ in *Oncorhynchus* and B_4^{1s} in *Salvelinus* or cathodal homotetramers $B_4^{1\circ}$ in Oncorhynchus and B_4^{2s} in *Salvelinus* respectively. Although the position of intermediate heterotetramer showed the same electrophoretic mobility in both genera, the electrophoretic mobility of $B_4^{2\circ}$ was not as anodal as B_4^{1s} and $B_4^{1\circ}$ was not as cathodal as B_4^{2s}. On the other hand, masu salmon presented a unique LDH-B system consisting of only one broad and strongly stained band which had almost the same electrophoretic mobility to $B_4^{2\circ}$ as the other *Oncorhynchus*.

These results indicated the allelic difference in Ldh-B_2 locus between masu salmon and Salvelinus species (Whitespotted char and dolly varden char) as well as the allelic difference in Ldh-B_1 locus between masu salmon and the other *Oncorhynchus* species. Such differences in the LDH-B isozyme system between masu salmon and the other salmonids will provide useful gene markers to investigate the activation time of the paternally derived gene.

Developmental analyses

As shown in Fig. 2, chum salmon demonstrated five banded isozyme pattern of the LDH-B system having binomially distributed staining intensity during the earlier stages of embryogenesis. This initial isozyme pattern had persisted until 13 days after fertilization and had changed to the other isozyme pattern with the dominance of $B_4^{2\circ}$ homotetramer at 16 days after fertilization. Muscle specific isozymes were barely detected from 28 days after fertilization.

In masu salmon, no isozymic change was observed and only one band belonging to the LDH-B system was detected during the embryogenesis before the appearance of heterotetramers between the LDH-B and LDH-A systems at around 28 days after fertilization (Fig. 3).

While in whitespotted char, a four banded isozyme pattern with the dominance of LDH-B^1 subunit was observed at least untill 11 days after fertilization (Fig. 4). At 14 days after fertilization, cathodal B_4^{2s} homotetramer was added to the LDH-B system (Fig. 4). Isozymic bands resulted from the gene activation responsible for the LDH-A system were faintly detected from 23 days after fertilization.

Developmental changes of LDH isozyme patterns in the hybrid between masu

salmon ♀ and whitespotted char ♂ are shown in Fig. 5. The isozyme pattern in the earlier stages from 1 to 11 days after fertilization was complete maternal masu salmon type of one isozymic band in the LDH-B system, but the appearance of new isozymes containing paternal LDH-B^{2s} subunit became detectable from 14 days after fertilization. The additional muscle specific isozymes faintly appeared from 26 days after fertilization.

Such a persistence of maternal isozyme during earlier stages of development and an appearance of new isozymes containing paternally derived LDH-B^{2s} subunit were also observed in the embryogenesis of the other intergeneric hybrid combination of masu salmon ♀ × dolly varden char ♂ (Fig. 6). In this hybrid between female masu salmon and male dolly varden char, the isozyme containing subunit B^{2s} originating from paternal dolly varden char first appeared from 13 days after fertilization (Fig. 6). As shown in Fig. 7, masu salmon ♀ × coho salmon ♂ hybrid demonstrated the maternal one banded pattern until 10 days after fertilization. A new isozyme containing paternal LDH-B^{1o} subunit appeared also from 13 days after fertilization at the same position to $B_3^2B_4^1$ heterotetramer of coho salmon (Fig. 7). A four banded pattern of the LDH-B system with a dominance of B^{1s} subunit had been revealed throughout the whole embryogenesis of hybrids between female whitespotted char and male masu salmon (Fig. 8). In this hybrid, there was not an isozymic change as those which occurred in the development of whitespotted char where the initial 4 banded isozyme pattern with the dominance of B^{1s} subunit was substituted for the five banded pattern. Heterotetramic bands between subunits of the LDH-B system and those of the LDH-A system were detected from 26 days after fertilization.

These evidences show that both the changes in isozymic patterns and the new appearance of isozymes containing paternal subunit occurred during a period from 13 to 16 days after fertilization. The morphological observations of the embryos revealed that the isozymic changes or new appearance of paternal subunit occurred at almost the same stage of the enclosure of the blastopore and the tail bud formation in all cases examined (Figs. 9, 10 and 11). An additional appearance of isozyme containing muscle specific LDH-A subunit may occur around the time of the eyed stage in every experimental embryos. No eye specific isozyme was recognized in these experiments during the period from fertilization to hatching.

Discussion

The present study showed a reversal in the appearance of liver or heart specific isozyme pattern of LDH-B between *Oncorhynchus* and *Salvelinus* species. This strongly suggests that the most anodal isozyme in the LDH-B system of *Oncorhynchus* will be the homotetramic product of Ldh-B_2 locus, with the cathodal one being the homotetramic product of Ldh-B_1 locus. While in the case of *Salvelinus* the anodal one in the LDH-B system will be of Ldh-B_1 locus, with the cathodal one being of Ldh-B_2 locus. It was proven, however, in this present study that there was only a slight difference in the electrophoretic mobility between B_4^{2o} and B_4^{1s} or B_4^{1o} and B_4^{2s} homotetramer. On the contrary, the LDH-B system of masu salmon

gave a one banded isozyme at about the same position as the LDH-B_4^{2c} homotetramer. In regards to this unique LDH-B system of masu salmon, Utter *et al.*[9] interpret that the homotetramic product of Ldh-B_1 locus had almost the same electrophoretic mobility as that of Ldh-B_2 locus, rather than the inactivation or deletion of Ldh-B_2 gene locus. Consequently, it was demonstrated that there is an allelic difference in Ldh-B_1 locus between masu salmon and the other *Oncorhynchus* species, and also another allelic difference in Ldh-B_2 locus between masu salmon and two Japanese *Salvelinus* species. These allelic differences between masu salmon and the other salmonid species provided useful gene markers in the developmental genetic analyses on the activation of paternal gene on Ldh-B_1 and -B_2 loci in the hybrid genome.

In the intergeneric combination of masu salmon ♀ × whitespotted char ♂ and masu salmon ♀ × dolly varden char ♂, LDH isozyme during earlier stages was of maternal masu salmon type until the tail bud stage. Thereafter, paternal B^{2s} subunit occurred. Also in the hybrid of masu salmon ♀ × coho salmon ♂, only a maternal LDH isozyme pattern was observed in earlier embryogenesis before the tail bud stage, and the isozyme containing paternal B^{1c} subunit occurred at the tail bud stage.

Similar phenomena for several isozyme have been reported in other teleost species.[1–7] There is some experimental evidence concerning a repression of embryonic genome in earlier embryogenesis of teleosts[10] and a presence of maternal enzyme even in nucleo-cytoplasmic hybrid amphibian embryos without maternally derived genome.[11,12] These have been interpretted as the contribution of the cytoplasmic stable enzyme which is synthesized during oogenesis and stored in the egg cytoplasm. This might be true in salmonids; the initial LDH isozyme pattern observed in the stages before tail bud formation may be the stable products synthesized during oogenesis.

It has been proven from hybrid experiments between masu salmon and the other *Oncorhynchus* or *Salvelinus* species that the activation of paternal alleles both on Ldh-B_1 and -B_2 loci occurred at the same stage of development in each hybrid. The Ldh-B_1 and -B_2 loci may be programmed in the embryonic genome to be activated at almost the same tail bud stage. These evidences suggest that the first activation of embryonic genome responsible for syntheses of LDH-B^1 and -B^2 subunit may start from the stage characterized with the enclosure of blastopore and the formation of the tail bud. Therefore, it may be possible to regard the tail bud stage as the turning point between the contribution of egg cytoplasmic LDH and the genomic function in the biochemical differentiation in salmonid embryo.

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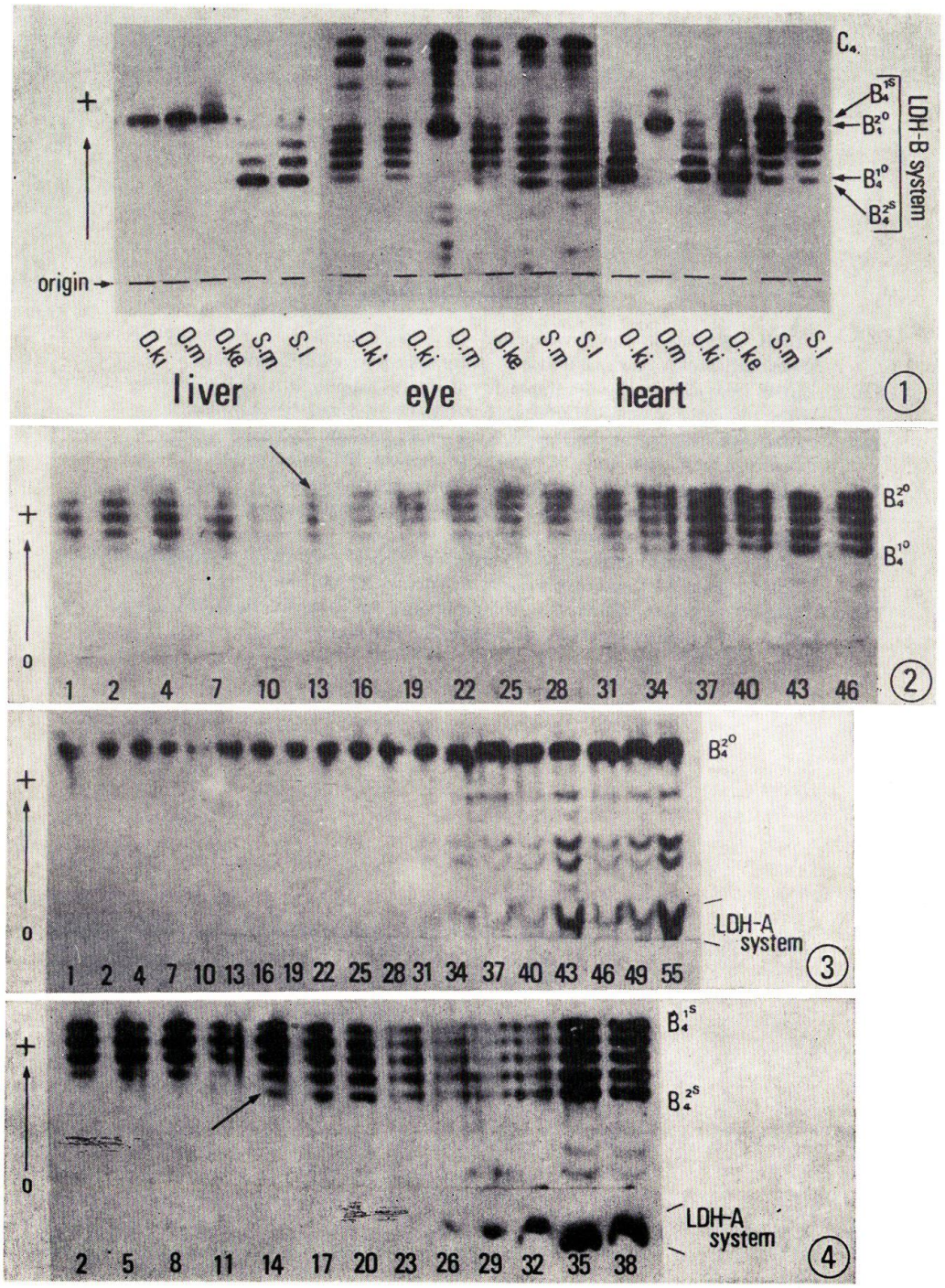
Explanations of Plate I

Fig. 1. Lactate dehydrogenase (LDH) isozyme patterns observed in eyes, hearts and livers among 5 species of salmonid fishes. O.ki; coho salmon (*Oncorhynchus kisutch*), O. ke; chum salmon (*O. keta*), O. m: masu salmon (*O. masou*), S. l: white-spotted char (*Salvelinus leucomaenis*), S. m; dolly varden char (*S. malma*).

Fig. 2. LDH isozymes in the development of chum salmon during a period of 1 to 40 days after fertilization. Arrow indicates the dominance of $B_4^{2^O}$ homotetramer from 13 days after fertilization.

Fig. 3. LDH isozymes in the development of masu salmon during a period of 1 to 55 days after fertilization.

Fig. 4. LDH isozymes in the development of whitespotted char during a period of 2 to 38 days after fertilization. Arrow indicates a new appearance of $B_4^{2^S}$ homotetramer.



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Explanations of Plate II

Fig. 5. LDH isozymes in the development of masu salmon ♀ × whitespotted char ♂. Arrow indicates a new occurence of paternally derived B^{2S} subunit.

Fig. 6. LDH isozymes in the development of masu salmon ♀ × dolly varden char ♂. Arrow indicates a new occurence of paternally derived B^{2S} subunit.

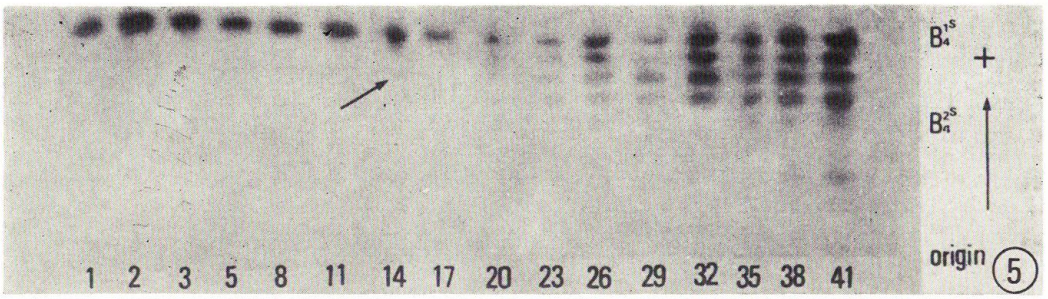
Fig. 7. LDH isozymes in the development of masu salmon ♀ × coho salmon ♂. Arrow indicates a new occurence of paternally derived B^{1O} subunit.

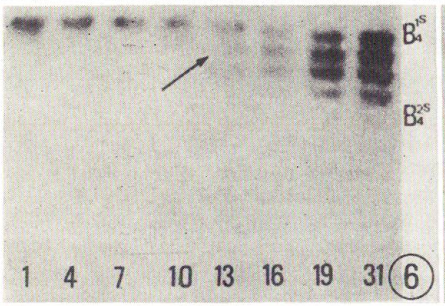
Fig. 8. LDH isozymes in the development of whitespotted char ♀ × masu salmon ♂.

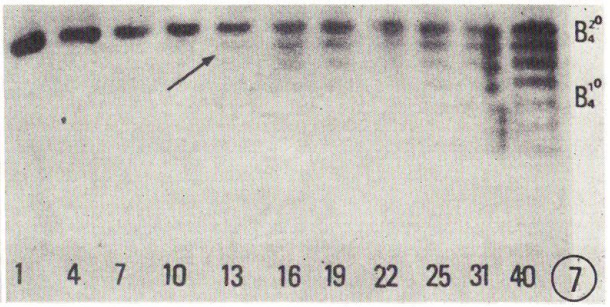
Fig. 9. Embryo of whitespotted char at 13 days after fertilization. × 20. L; lens, AV; auditory vesicle, S; somite, T; tail bud.

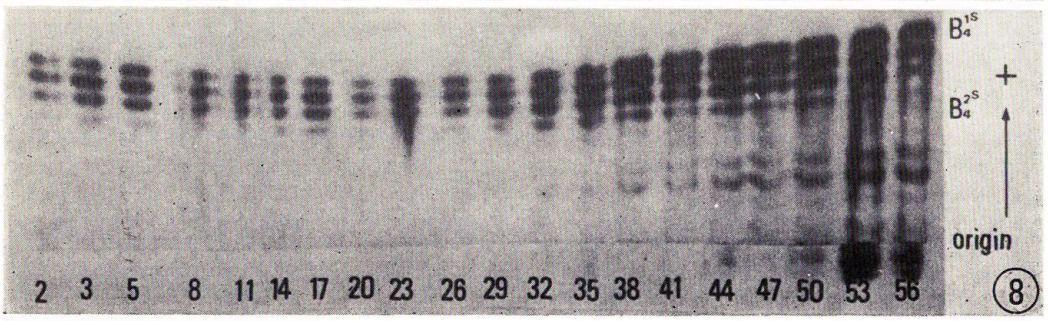
Fig. 10. Embryo of masu salmon ♀ × dolly varden char ♂ at 13 days after fertilization. × 20, L; lens, AV; auditory vesicle, S; somite, T; tail bud.

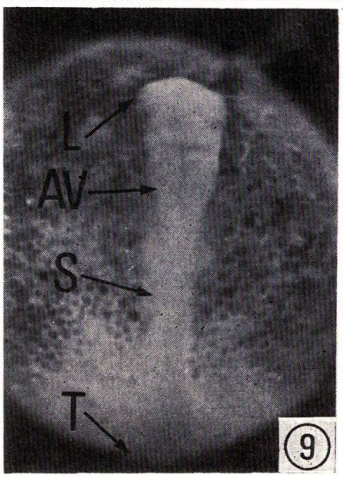
Fig. 11. Embryo of masu salmon ♀ × coho salmon ♂ at 13 days after fertilization, × 20. L; lens, AV; auditory vesicle, S; somite, T; tail bud.

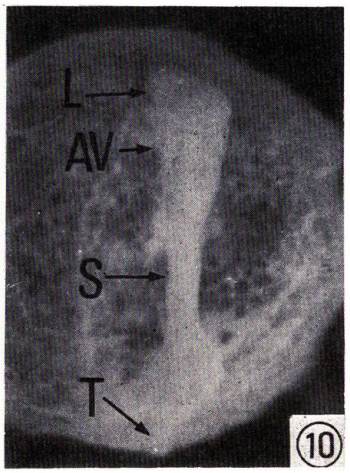


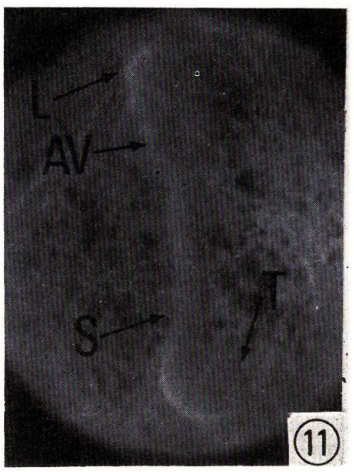












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