A Method of Lipid Injection into a Fish Egg

In order to study the behaviour of carotenoid in the medaka (*Oryzias latipes*) embryo[1,2] a method of carotenoid injection into a fish egg is devised. The method is also applicable for sex hormone and other lipids.

Instruments. The injection is made by a micropipette which has a fine tip and rubber nipple. Lipid which has been dissolved in solvent is pushed out of the pipette by pressing the rubber nipple. A bigger tip injures the egg and a smaller one decreases the injection speed. The injection can be made by hand under a binocular microscope. However, the injection is more successful when the pipette is fixed on a manipulator. In order to put oil in which lipid has been dissolved into the tip of the injection pipette, an auxiliary pipette is used. The long capillary of the auxiliary pipette first sucks the oil up, and the oil is then transferred into the injection pipette. Eggs must be fixed during injection. A slide on which a small piece of mosquito net is fixed is convenient, as a fish egg can be fixed in one of the holes.

Solvent. Lipid, such as carotenoid and sex hormone, is dissolved in the solvent before injection. The selection of solvent is important because the lipid must be dissolved completely in the solvent and the fish larvae must be able to absorb the injected solvent, and also it must not be poisonous. Several solvents, such as olive oil, rape seed oil, linseed oil, castor oil, alcohol, ligroin, liquid paraffin and water, were tested. Alcohol and ligroin were poisonous. Fish larvae could not absorb liquid paraffin. Rape seed oil and linseed oil oxidized the dissolved lipid rapidly. Olive oil was a good solvent for the lipids which do not have polar groups, such as carotene, and fish larvae absorbed it well (Table). Castor oil has hydroxy fatty acid in its molecule and it is a good solvent for the lipids which have polar groups, such as various xanthophylls. Although castor oil is somewhat poisonous, the mixture of olive oil and castor oil is scarcely poisonous and it is a good solvent for the lipids with polar groups. The fish larvae can absorb the mixture of one volume of olive oil and one volume of castor oil (Table).

Even the olive oil, which is fairly stable, oxidizes dissolved lipid slowly. A very small amount of vitamin E added to the oil prevents oxidation. Lipid is dissolved in a small amount of oil before injection as follows: one drop of oil is dropped into ethyl ether or petroleum ether (boiling point 35–40°C) solution of the lipid. Then the ethyl ether or petroleum ether is evaporated completely over hot water and under reduced pressure.

Absorption of injected oil by larvae

Solvent	Larva	Volume of injected oil[a] Newly hatched	10 days after hatching	Volume of absorbed oil[a]
Olive oil	A	$15.1 \cdot 10^7 \mu^3$	$1.9 \cdot 10^7 \mu^3$	$13.2 \cdot 10^7 \mu^3$
	B	$6.1 \cdot 10^7 \mu^3$	$0.8 \cdot 10^7 \mu^3$	$5.3 \cdot 10^7 \mu^3$
	C	$3.7 \cdot 10^7 \mu^3$	$0.1 \cdot 10^7 \mu^3$	$3.6 \cdot 10^7 \mu^3$
	D	$3.7 \cdot 10^7 \mu^3$	$0.2 \cdot 10^7 \mu^3$	$3.5 \cdot 10^7 \mu^3$
	E	$1.9 \cdot 10^7 \mu^3$	$0.0 \cdot 10^7 \mu^3$	$1.9 \cdot 10^7 \mu^3$
Olive oil:castor oil = 1:1	F	$37.2 \cdot 10^6 \mu^3$	$18.8 \cdot 10^6 \mu^3$	$18.4 \cdot 10^6 \mu^3$
	G	$18.8 \cdot 10^6 \mu^3$	$8.0 \cdot 10^6 \mu^3$	$10.8 \cdot 10^6 \mu^3$
	H	$16.3 \cdot 10^6 \mu^3$	$2.5 \cdot 10^6 \mu^3$	$13.8 \cdot 10^6 \mu^3$
	I	$4.2 \cdot 10^6 \mu^3$	$0.3 \cdot 10^6 \mu^3$	$3.9 \cdot 10^6 \mu^3$
	J	$3.3 \cdot 10^6 \mu^3$	$0.0 \cdot 10^6 \mu^3$	$3.3 \cdot 10^6 \mu^3$

[a] Volume is calculated from the diameter of injected oil drop.

Lipids dissolve in water by making complexes with some surface active agents. The aqueous solution of lipid-Tween 80 complex is scarcely poisonous when it is injected into a fish egg. The injected complex diffuses rapidly in yolk.

Injection. The egg of the medaka is especially convenient material for the injection (Figure) because it is transparent and the artificial fertilization[3] can easily be made in a balanced salt solution. The fertilization membrane is soft within a few minutes after fertilization and injection at this stage is successful, whereas injections at later developmental stages result in failure. All injections at animal pole, vegetal pole and other points between these poles are equally successful. However, injection into the surface area is not good. The operated eggs can be allowed to develop in the balanced salt solution without antibiotics. About 80% of the operated eggs are able to develop normally if all the conditions are good.

The quantitative injection is done by the following formula.

$$R = 12.4 \times \sqrt[3]{\frac{A}{C}}$$

where R is the diameter of injected oil (mm), A the quantity of lipid to be injected (μg), and C the concentration of lipid in injection solution (μg/ml). If A and C are known, R can be calculated. The injection may be stopped when the diameter of injected oil drop becomes R. Several experiments with olive oil suggest that the maximum of R is one half of the egg diameter[4].

A medaka egg at the stage of four cells. Arrow shows the injected olive oil stained by sudan III (about × 20).

Zusammenfassung. Mittelst Mikropipette von einem Spitzendurchmeser 15–20 μ werden Lipide in Fischeier injiziert. Die leichtere Löslichkeit der Lipide wird mit Olivenöl oder einem Gemisch Olivenöl/Rizinusöl erreicht. Unmittelbar nach der Befruchtung ist der Einspritzungserfolg am grössten.

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1 K. TAKEUCHI, Embryologia *5*, 170 (1960).
2 K. TAKEUCHI, Zool. Mag., in Japanese *71*, 21 (1962).
3 T. YAMAMOTO, Proc. Imp. Acad., Tokyo *15*, 267 (1939).
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