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Inducing Effects of the Killed Archenteron Roof on late Gastrula Ectoderm

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Using *Triturus pyrrhogaster* embryos the writer has demonstrated (1943) the existence of the regional difference of the capacities to induce the sensory organs within the archenteron roof; the prechordal plate induces both the nose and lens from the late gastrula ectoderm, and the anterior portion of the notochordal mesoderm induces the ear. The nose- and lens-inducing capacities of the prechordal plate become effective after the onset of gastrulation, but soon disappear in a comparatively early stage of development (middle gastrula). Nevertheless, if such a non-effective prechordal plate is transplanted with the fore-brain the nose can be induced frequently in the trunk region where it scarcely occurs by transplanting of the fore-brain alone. On the other hand, the anterior portion of the presumptive notochord taken from the upper blastopore lip has the capacity to induce the ear. This capacity is preserved till the later gastrula stage, and at this stage it is most effective at the anteriormost region of the notochord and gradually decreases posteriorly to be lost at a level immediately posterior to the fore-limb. But the ear-inducing capacity is nearly deprived by killing the archenteron roof at 60° C, and the pieces of the archenteron roof become effective by such treatment in inducing the nose and the lens no matter where they are taken from (KAWAKAMI, 1943, 1949).

The present experiments were performed in aim of studying the inducing mechanisms of the sensory organ by way of examining the effects of the archenteron roof which was treated with some chemicals.

Having the *Triturus* embryos in yolk-plug stage immersed for 17—20 hours in the chemicals of HNO_3 (2.5 %), H_2SO_4 (5 %), alcohol (100 %), aceton (100 %) and formalin (10 %), the archenteron roof and the blastopore lip were cut out as a cord and was washed through

several changes of water for 2.5—3 hours. Then, each cord was cut into seven pieces of equal size: the first piece corresponds to the fore-gut endoderm, the next two pieces to the prechordal plate and the other posterior pieces to the notochordal material. All of the pieces except the first were applied as inducing grafts. The operation was performed after the same method as had been adopted in the previous experiments to test inducing capacities of fresh archenteron roof (KAWAKAMI, 1943, 1944, 1949); namely, each of the inducing grafts were transplanted to the branchial region of the neurula and afterwards covered by the presumptive epidermis taken from another embryo almost through with gastrulation. The operated specimens were fixed at stage 42 (OKADA AND ICHIKAWA'S stage of the normal development of *Triturus pyrrhogaster*, 1948). When histologically examined the implants were found absorbed at this stage.

The results are shown in the table on page 99.

The nature of such inductions as the nose, lens, ear, neural tissue, notochord and muscle can be easily identified, but the inductions termed in the table as vesicle and sensory bud cannot determined histologically to which organs they are homologous.

Judging, however, from the structural figures, the former seems to be an abnormal or incomplete ear-vesicle or perhaps a part of the brain, and the latter to be a neural tissue or an abnormal nose.

At all events, the experimental results are interesting in the following four points: (1) The regional difference of the inducing capacities of the archenteron roof in fresh conditions disappears after the treatment with the chemicals. The ear-induction also be observed in the present case, though it rarely occure when the archenteron roof killed through heating is grafted. (2) The induction of the nose and the lens becomes of rare occurrance when the archenteron roof is treated with H_2SO_4, while the ear induction takes place frequently. Moreover, the muscle induction occurrs through the pieces of the acid-treated archenteron roof, and the one induced through the piece of the HNO_3-treated archenteron roof accompanies the notochord-induction. As the muscle and notochord are found apart from those of the host, it is obvious that these structures have arised from the foreign mesodermal material of host. (3) The inducing effects of the archenteron roof treated with alcohol and aceton notably different from those in the H_2SO_4-treatment, i. e, the formation of the nose and the lens takes place more frequently than in the latter case. The other characteristic features of these series are the simultaneous appearance of nose and lens

Table: The inductions through the pieces of archenteron roof treated with chemicals.

Grafts	Results / Chemicals	Available cases	Induction						
			Nose	Lens	Ear	Vesicle	Sensory bud	Neural tissue	Muscle
2nd piece	HNO_3	16	1		1		1	3	
	H_2SO_4	14			1	2			1
3rd piece	HNO_3	13	1		1×	1	2	3, 1×	
	H_2SO_4	14			3				1
4th piece	HNO_3	14				2		2	
	H_2SO_4	13			1×, 1		1×, 1	1×	
5th piece	HNO_3	15				1		2	1(+Not.)
	H_2SO_4	11		1×			1×		1
6th piece	HNO_3	13	1×	1×	1	2	1		
	H_2SO_4	13			1				
Blast. lip	HNO_3	9						1	
	H_2SO_4							1	
Total	HNO_3	80	3	1	3	6	4	11	1(+Not.)
	H_2SO_4	78	0	1	7	2	3	2	3

Grafts	Results / Chemicals	Available cases	Inductions						
			Nose	Lens	Ear	Vesicle	Sensory bud	Neural tissue	Muscle
2nd piece	Alco.	16		1		2			
	Acet.	14		1×	1	1	1×	1	
3rd piece	Alco.	15		1×, 1		1	1×, 1		
	Acet.	15		1×			1×, 1		
4th piece	Alco.	19		1×	2	1		1×, 1	
	Acet.	15		1×		1	1×, 1	1	
5th piece	Alco.	18	1×	1×				1	
	Acet.	12	1×, 1°	1×, 1°	1			1	
6th piece	Alco.	16	1×	1×	1	1		1	
	Acet.	21	1×, 1		1	2		1×. 1	
Blast. lip	Alco.	19	1	1	1				
	Acet.	11	1×, 1	1×, 1				1	
Total	Alco.	103	3	7	3	5	2	4	0
	Acet.	88	6	7	2	4	5	7	0

The marks of ×, ○ show the organs simultaneously inducted in one specimen.

or lens and sensory bud in a single specimen. In some of these specimens the induced nose and sensory bud partially differentiate into lens or lens fibers. (4) The archenteron roof treated with formalin turn out to be weak in inducing effects; only three inductions, one an

ear and the other two sensory buds, are observed in eighty seven available cases.

One of the principal objects of this study is to produce the specific effect of inducing one of the sensory organs by treating the archenteron roof with various chemical. Though in this angle the present results are not necessarily satisfactory, the differences between the inducing effects of the H_2SO_4-treated archenteron roof and those treated with alcohol or aceton respond to this purpose at least to certain extent; the ear induction is frequent in the former while the formation of the nose and the lens takes place frequently in the latter.

Summarizing the present results and other facts which have been obtained in the writer's works, he is inclined to consider that the ear inducing mechanism is entirely different from those of the nose and lens in quality.

The state of affairs seem, however, to be different with the developmental mechanisms of the lens, nose, fore-brain and eye-cup. The fresh eye-cup is capable of inducing the formation of the lens from the presumptive ectoderm, whereas the boiled one induces not only the eye-cup from the younger presumptive epidermis but also the lens from the ectoderm of the late gastrula (KAWAKAMI, in press). The anterior region of the prechordal plate induces the lens from the late gastrula ectoderm, but eye from the younger one. These facts lead the writer to the opinion that both lens and eye-cup are formed through the same inducing agents save for the difierence of intensity, and that the inducing effect of killed eye-cup is momentary but powerful to induce the eye formation from the younger ectoderm, while the inducing action of the fresh eye-cup not be strong enough to induce the eye formation but hold throughout comparatively long period to result in the lens formation. It may be also assumed that the inducing mechanism of the nose and the fore-brain is similar to the one mentioned above. Furthermore, the simultaneous induction of the nose and the lens through the dead tissues give the impression that there is much similarity between the inducing mechanism of the nose and the lens.

In any case, the point to be emphasized is that in considering the problems in relation to the differences in the inducing mechanism of these organs, the writer does not assume a single specific substance to each of these inductions but the specific physico-chemical system participating each inducing action.

Taking into consideration the facts that the induction of the ear and the mesodermal organs, in general, are of simultaneous occurrence,

the appearance of both ear and muscle in the specimens in which the H_2SO_4-treated archenteron roof has been transplanted, seems to be quite natural. But according to a recent results obtained by YAMADA (in press) claiming the formation of the notochord and the muscle after a brief exposure of the ventral mesoderm of the gastrula to a solution of ammonium, so a more plausible interpretation would be to attribute the formation of muscle and the notochord to the direct effect of the acid, left in the graft from washing, upon the presumptive mesodermal cells in situ.

Consulting the facts that not only the organizers but also some non-inducing tissues yield strong inducing effects after killing them by means of the various methods, the obvious loss of the effects to induce the sensory organs of the archenteron roof treated with formalin is peculiar, and may be worthy of further consideration. Nevertheless, as far as the present experiments are concerned the writer is not prepared to give any plausible explanation for it at present. The well-known fact that formalin and protein are combined into a particular compound might throw some sugestion to the question.

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