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Experiments on the Amphibian Mesectoderm, with Special Reference to the Cartilage-Formation

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With 25 Text-figures

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I. Introduction

STONE's experiments (1922–1933) bearing directly upon the development of visceral cartilages in amphibians have brought us the following interesting results: 1) That most of the visceral cartilages in *Amblystoma punctatum* and *Rana palustris* are formed from the mesectoderm descended from the neural fold, 2) that the mesectoderm can differentiate into cartilage even in the heterotopic mesentoderm, 3) that the basibranchium is of double origin, the first basibranchium, according to his nomenclature, being derived from the mesectoderm and the second basibranchium from the mesentoderm. RAVEN (1932–1935) affirms these facts of development generally in *Amblystoma mexicanum*.

However, in these previous experiments the formation of cartilages was found when the transplant happened only to be placed in the mesentodermal environment and it is not quite conclusive whether the mesectoderm gives rise directly to cartilage or whether it only induces cartilage from the neighbouring mesentoderm. In one of RAVEN's experiments (1933), indeed, transformation into cartilage took place in a xenoplastic transplantation of the mesectoderm of *Rana fusca* into the blastocœl of *Triton taeniatus*. I undertook the present experiments at the suggestion of Prof. Yô K. OKADA to verify this part of RAVEN's findings by transplanting the same embryonic tissue into a brain-cavity or into an endoderm where the cartilaginous formation had not been proved.

In addition, the development of visceral cartilages was studied, especially concerning the problems, 1) when the mesectodermal tissue is determined to give cartilaginous tissue, 2) what cartilage of the branchial basket is derived from what group of mesectoderm, 3) whether the second branchial cartilage takes share in the formation of the hypobranchial plate, 4) whether or not the basibranchium is of double origin and 5) whether the mesectoderm is the origin of the auditory capsule.

Before going farther I take this opportunity to express my hearty thanks to Prof. Yô K. OKADA for his kind suggestions and encouragement as well as much valuable criticism throughout the whole course of the work.

II. Normal development of the cranial mesectoderm

Investigation was done mostly on *Rana japonica* and when experiments were made, *Hynobius nebulosus* was used as well. The

frog *Rana japonica* begins to spawn in late Junuary. Its eggs are large enough for the experiments, and during development, are less likely to be attacked by molds due to the cold weather. The mesectoderm of this species is very characteristic and clearly differentiated from other cell layers. When the overlying ectoderm is removed in a proper stage of development, it can easily be recognized as dark pigmented masses, making a distinct contrast to the underlying mesentoderm which is less pigmented and rather whitish gray in colour. Besides this colour difference, both kinds of mesoderm are more distictly divided in sections; namely the cells of the mesentoderm are larger and laden abundantly with large yolk granules and more strongly stainable with eosin. Those of the mesectoderm are smaller and contain a small quantity of fine yolk granules and are less stainable.

In the following the description is first concerned with the normal development of the mesectoderm, since the knowledge of it is a great help towards understanding the results of the experiments.

Stage 1. It represents the period when the yolk-plug is reduced to a slit-like depression, i. e., a late gastrula with the medullary plate still very faintly defined. In this stage the medullary plate is nothing but a mere thickening of the ectoderm, mesectodermal cells being contained in it. But in sections we observe in the plate two distict kinds of cells; those of the median part, which later become the neural tube, are columnar in shape and regular in arrangement, whereas those of the periphery are polygonal and massive. This last type of cells represents the mesectoderm. Fig. 2, *a* shows a slightly advanced stage, and the demarcation between two kinds of cells is quite conspicuous.

In the external appearance, the primary neural folds appear a little later and thus the mesectodermal part becomes clearly separated from the median part as in fig. 1, *a*. The fold is wide in the cranial region and this is the only part concerned with the formation of the visceral cartilages.

Stage 2. Along and inside of the primary fold, the secondary fold makes its appearance with a crescent-shaped depression between two folds in the cephalic region (fig. 1, *b*); the new fold is converted in due course into the neural tube. Already in this stage the prospective optic region is indicated as a pair of crescent-shaped areas near the anterior end of the neural plate.

The primary neural fold begins to divide on each cephalic side

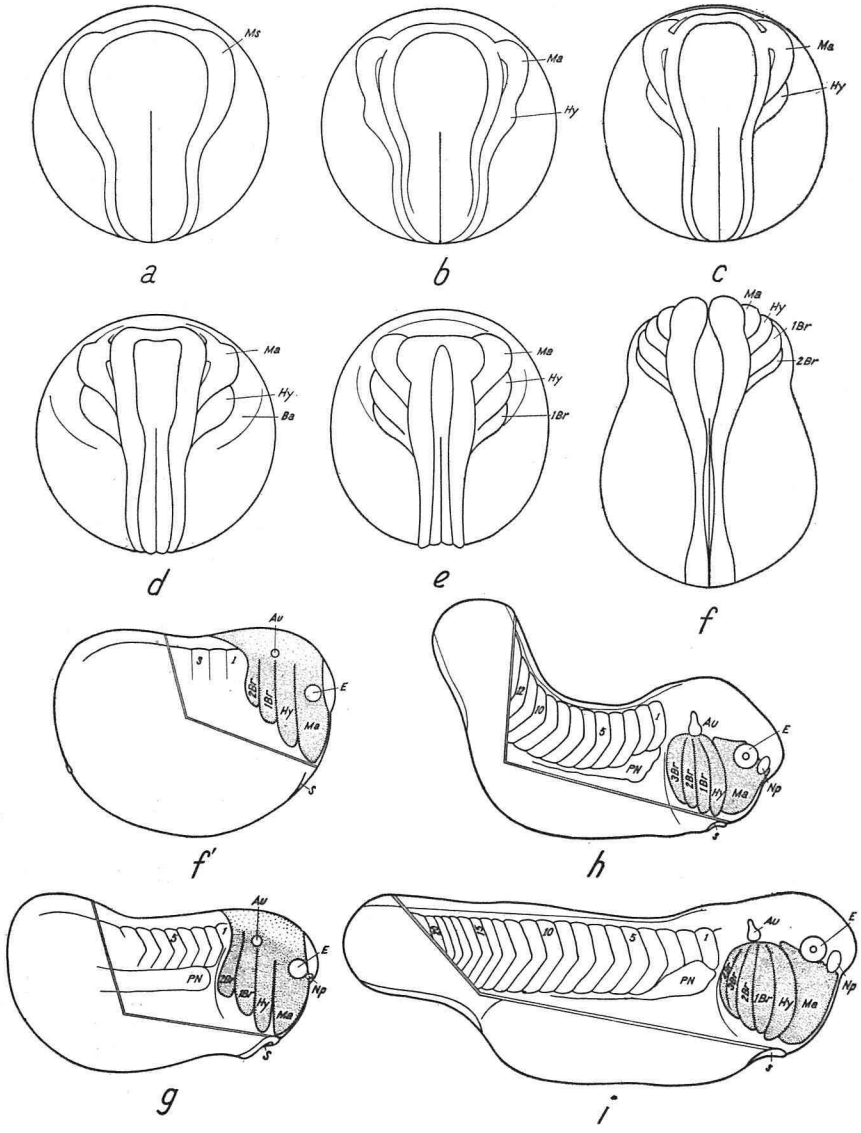

Fig. 1. Normal stages of *Rana japonica,* showing the development of the cranial mesectoderm. In the last four figures the overlying ectoderm is denuded in order to expose the mesectoderm (pointed) to the view. *Au,* auditory placode or vesicle; *Ba,* branchial arch; 1-4 *Br,* first to fourth branchial mesectoderm; *E,* eye; *Hy,* hyoid mesectoderm; *Ma,* mandibular mesectoderm; *Ms,* mesectoderm; *Np,* nasal placode; *PN,* pronephros; *S,* sucker; numerals represent the order of myotomes.

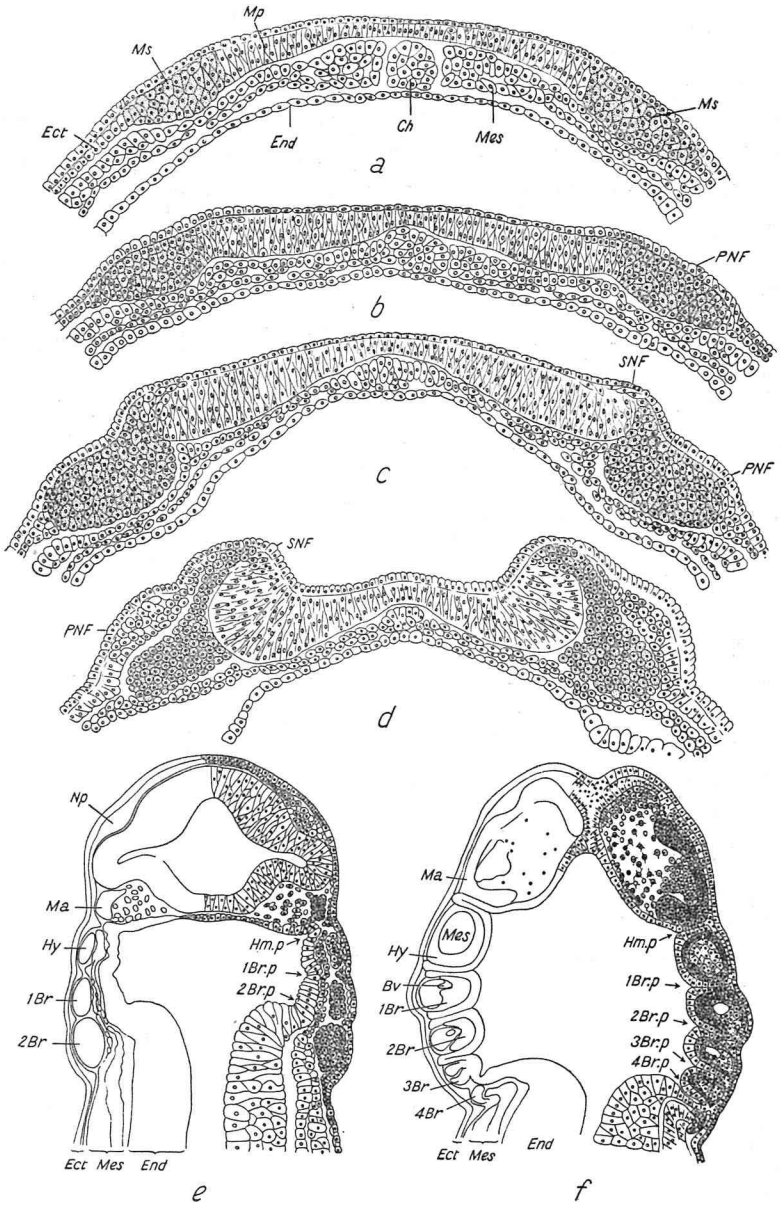

Fig. 2. Camera-drawing, showing the development of the mesectoderm in sections. *a–d,* cross sections through the broad part of the medullary plate; *e–f,* frontal sections of the head. *a,* stage 1; *b,* a slightly advanced stage; *c,* stage 2; *d,* stage 3; *e,* stage 7; *f,* stage 9. 1–4 *Br. p,* first to fourth branchial pouches; *Bv,* blood vessel; *Ect* ectoderm; *End,* endoderm; *Hm. p,* hyomandibular pouch; *Mes,* mesentoderm; *Mp,* medullary plate; *PNF,* primary neural fold; *SNF,* secondary neural fold; other letters are as before.

into two parts in the broadest portion by a shallow indentation to extend anterolaterally. The anterior division represents the mandibular mesectoderm and the posterior division, the hyoid. The lateral mesectodermal part of the neural plate is more clearly distinguished from the median medullary part in cross section than in superficial observation, especially in the cephalic region (fig. 2, *c*). Separation of the mesectoderm from the overlying epidermis is not yet complete. Some part of the epidermis being rolled later in the neural tube when this is formed, the underlying mesectoderm migrates laterally in the opposite direction and is not concerned with the tube formation as in the case of urodeles.

Stage 3. In this stage the secondary neural folds approach nearer than before and separation of the mandibular and hyoid groups becomes clear (fig. 1, *c*). The two groups are now elongated somewhat anteroventrally, and between the mandibular group and the head fold is made a space which is seen as a depression from the surface. The stomodeal invagination takes place afterwards in the middle of this part.

Fig. 2, *d* shows a cross section through the area where the neural folds are most apart; the mesectoderm migrates downwards on either side of the neural plate in the shape of an Indian club, the handle of which does not yet detach itself from the margin of the plate.

Stage 4. No further differentiation are involved in the development of the mesectoderm except its further downgrowth. But it is a special feature of this stage that the branchial arch appears, though faintly, to the posteroventral side of the hyoid mesectoderm (fig. 1, *d*).

Stage 5. The most characteristic feature of this stage is the appearance of one more division of the mesectoderm, i. e., the first branchial group on the branchial arch. It is still small and extends ventrolaterally far less than the preceding two groups (fig. 1, *e*). Internally the mesectoderm almost detaches itself from the seat of origin. The embryo is now elongated a little in the antroposterior direction, and the fore-brain is swelled out on both sides as optic vesicles.

Stage 6. The embryo measures 2.5-2.8 mm. in length. The neural folds are almost closed along the whole length, except the cranial region (fig. 1, *f* and *f'*). When the ectoderm in the latter region is removed the optic vesicle is seen surrounded by the mandibular mesectoderm. The nasal and auditory rudiments are also discernible as low and small placodes. The first is located in front of

and ventral to the optic vesicle closely attaching to the fore-brain, checking the extention of the mesectoderm beyond this point. The second is found on the upper part of the hyobranchial groove separating the hyoid and branchial groups of the mesectoderm. Four somites are counted, as a rule, when the ectoderm is denuded from the side of the body. The rudiments of suckers appear. As the characteristic feature of this stage may be mentioned the appearance of the second division of the branchial mesectoderm.

Internally the hyomandibular pouch is produced on each side of the fore-gut; other pouches are yet imperfect, the mesentoderm remaining in the state of two-cell thickness in continuation, i. e., not broken into pieces corresponding to each visceral arch respectively.

Stage 7. The embryo measures about 3.5 mm. in length; the posterior end becomes slightly swelled out to the dorsal side (fig. 1, *g*). The neural folds which were closed in the previous stage fuse completely along the whole length. Seven pairs of somites are formed, below which the pronephros appears as a longitudinal prominence on each side of the body. The optic vesicles become discernible from the surface. The suckers and a stomodeal depression are clearly distinct. The positions of the ears are indicated on the upper part of each hyobranchial depression as dark pigmented spots. In sections the auditory placode is converted into a vesicle almost split off from the seat of its origin. The lens formation has not yet commenced and the nasal placode is not yet separated from the fore-brain. The first to the third branchial pouches are in the course of formation in addition to the hyomandibular pouch produced in the previous stage. As to the mesectodermal differentiation the upper part of the mandibular division thins out into a layer which would give rise to the mesenchymal tissue of this region. The mesectoderm in the branchial region tends to divide into each corresponding arch. The mandibular mesectoderm is completely isolated from the rest by the attachment of hyomandibular pouch and the corresponding thickness of the ectoderm.

Stage 8. The embryo is in the tail-bud stage and about 4.2 mm. in length (fig. 1, *h*). There are usually twelve pairs of somites. The nasal pits are indicated on the surface as dark pigmented spots. The lens placode projects into the optic cup, without, however, entirely detaching from the ectoderm. The ear vesicle is pear-shaped and sinks deeply inwards. The suckers are functional and secrete adhesive mucus. The most characteristic in this stage is the formation

of the third division of the branchial mesectoderm on the corresponding arch. Other divisions of the branchial mesectoderm partly wrap the mesentoderm from both anterior and posterior sides until they are situated next to the endoderm.

Stage 9. The embryo is about 5.7 mm. in length, and the tail elongates towards the longitudinal axis of the body. The gill rudiment makes its appearance as a conical process on the first branchial arch (fig. 1, *i*). The heart has been formed but as yet there is no sign of beating. The specific feature of this stage is the splitting off of the fourth division of the branchial mesectoderm from the third by the appearance of the fourth branchial pouch and by the vertical thickening of the ectoderm against the corresponding pouch from the outer side (fig. 2, *f*). The greater part of the other visceral mesectoderm comes together beneath the mesentoderm to become cartilaginous tissue, while the part that remains on the mesentoderm takes share in the formation of the mesenchyme of the gills. In this stage the blood vessels make their first appearance in the first and second branchial arches.

As development goes further, the mandibular and hyoid groups of the mesectoderm gradually extend downwards until they meet the partner on the opposite side at the median line of the body. A bar is produced from the point of union of the hyoid groups in the caudal direction to lay down the anlage of the first basibranchium, to which the first pair of the branchial mesectoderm attach from each side. The ventral portion of this mesectoderm spreads itself posteriorly to form the hypobranchial plate. The other pairs of the branchial mesectoderm extend also downwards to the ventromedian line and fuse with the broadest part of the first pair just mentioned. The second pair only contribute to some extent to the formation of the plate, but not the others. The second basibranchium is added last to the caudal tip of the first like the root-cap of a plant. Thus the plan of the branchial basket is laid down.

III. Experiments

A) *Transplantation experiments of the cranial mesectoderm*

a) *Transplantation into the brain-cavity*

1) *Methods:* Operation was performed by the use of a glass needle and a hair-loop in 1/3 RINGER's solution or in HOLTFRETER's modified mixture.

The mesectoderm to be grafted was obtained from embryos in

stage 6 or 7. The ectoderm of the head was incised on the lateral side in U-shape and turned dorsally around the uncut border to expose the mesectoderm to the view. After removing, care being taken not to contain the mesentoderm in it, the mesectoderm was inserted into the brain-cavity of another individual in the same or a little advanced stage. The wound where the graft was inserted was pressed together by a glass load. The wound being healed, the embryo was transferred to a glass container and reared separately till the external gills were just covered by the operculum, when the cartilage was formed.

The experimental specimens were preserved using BOUIN's mixture as a fixative. Sections were cut 10–12 micra in thickness and stained with methylen blue as well as with DELAFIELD's hematoxylin either with or without eosin as a counter staining. Sometimes staining with borax carmin before embedding was applied.

2) *Homoioplastic transplantation :* In this series of experiments, *Rana japonica* was used as both donor and recipient. The transplanted region was sometimes indicated on the surface as a small

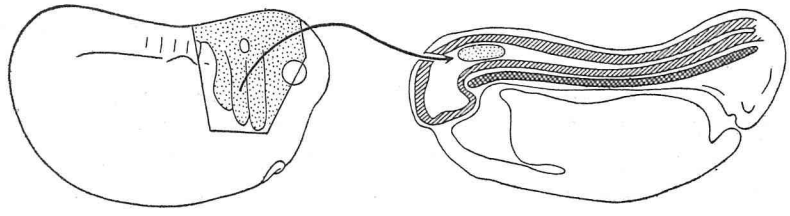

Fig. 3. Schema, showing the homoioplastic transplantation of the cranial mesectoderm into the brain-cavity.

elevation caudal to the optic region between two eyes. When the operation was unsuccessful, the graft was often found to be located above the brain. Those cases are not useful for the present purpose. However they are important because they prove that the mesectoderm, when it is in the mesenchyme, can differentiate more satisfactorily than when it is in the brain, an abnormal position. In the brain-cavity chondrification of the graft was more or less retarded. At any rate this fact is sufficient for us to prove the direct transformation of the mesectoderm into cartilage. In fig. 4 in which some representative examples are shown, cartilage is found in the brain-tissue in 3 cases and in one case it is floating freely in the cavity without attaching anywhere. Since our knowledge does not allow us to interpret the brain's ability to give rise to the cartilage or cartilaginous tissue, we can not but believe the product in the figures

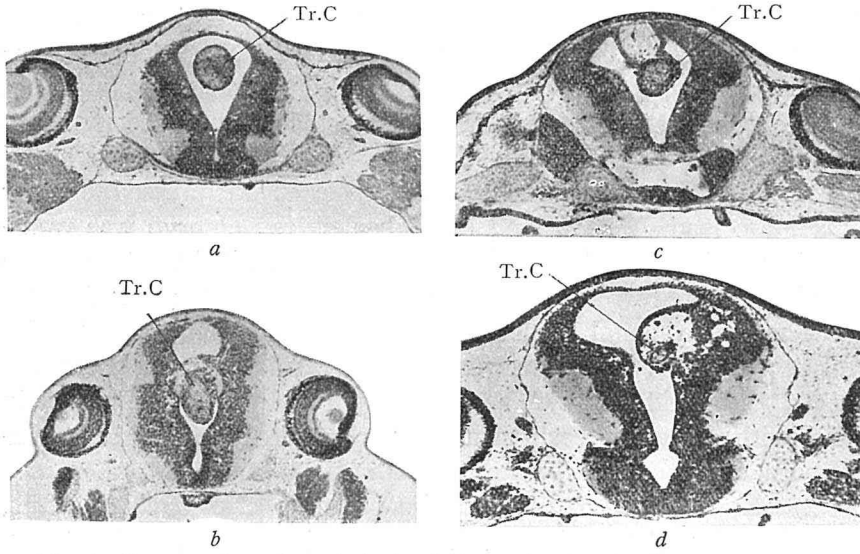

Fig. 4. Cross sections through the level of eye, showing the direct transformation of the mesectoderm into cartilage in the brain-cavity. In *a* the cartilage is floating in the cavity, while in other figures it is located in the brain tissue. *a,* RinB 78 (19/III-27/III, 1936); *b,* RinB 34 (27/III-5/IV, 1935); *c,* RinB 85 (19/III-31/III, 1936); *d,* RinB 69 (19/III-31/III, 1936). Tr. C, transplanted cartilage.

is the result of the direct transformation of the graft. Especially fig. 4, *a* gives this impression more emphatically, for the cartilage here floats in the cavity and the brain is normal in all appearance.

3) *Xenoplastic transplantation:* To show the potency of cartilaginous formation of the mesectoderm more definitely, xenoplastic transplantation was resorted to, taking *Rana* as donor and *Hynobius* as recipient. But unfortunately the grafts in the brain-cavity were all degenerated and became pigmented vestiges. In some cases, however, a piece of cartilage was found to be buried in the mesenchyme above the brain (fig. 5). It is no doubt formed from the graft, because its structure has all the appearance of *Rana* origin which is easily discernible from the comparison of the cartilage between two species. RAVEN ('33) obtained also a similar result in the combination of *Rana fusca* and *Triton taeniatus*,

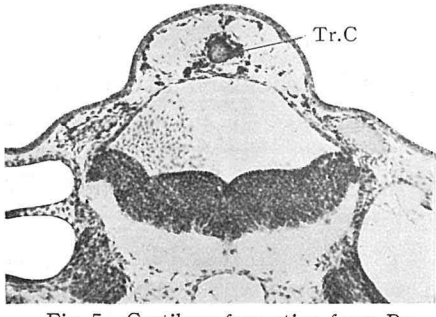

Fig. 5. Cartilage-formation from *Rana* mesectoderm in *Hynobius* mesenchyme. RHinB 31 (22/III-7/IV, 1936).

as already mentioned in the introduction. The results of RAVEN's experiment as well as mine clearly show the possibility of direct transformation of the mesectoderm into cartilage even in the xenoplastic surroundings. Reciprocal transplantation was not tested.

b) *Transplantation into the endodermal environment*

A piece of the mesectoderm was inserted into the gastrocœl or yolk mass of another embryo, the process of experiments being illustrated in fig. 6. As far as is known endoderm does not give rise to cartilage as the brain-tissue in the preceding experiment, so that

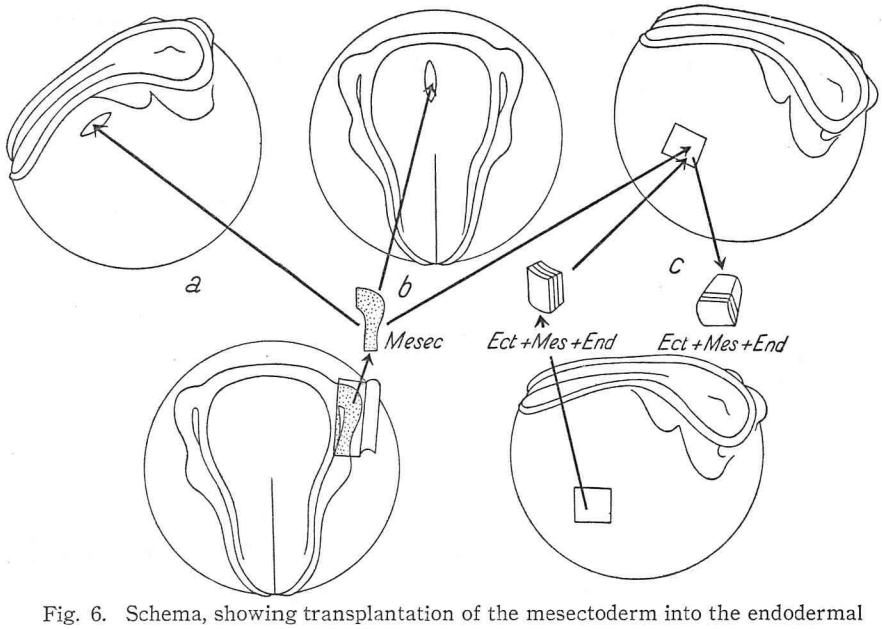

Fig. 6. Schema, showing transplantation of the mesectoderm into the endodermal environment. *a* and *b*, transplantation into gastrocœl; *c*, transplantation into yolk mass.

it will provide another proof of chondrification of the mesectoderm, if we find a cartilage or cartilaginous tissue after such transplantation. When the graft was buried in the endodermal tissues, the chondrifying process seems to be arrested and no satisfactory results were obtained (fig. 7, *a*). But when the same transplantation was done along with the underlying mesentoderm, the graft was able to differentiate into cartilage (fig. 7, *b*). This fact naturally leads us to suppose that the mesentoderm affords a condition for the mesectoderm to give rise to the cartilage, though the conclusion is not quite definitive on account of the small number of cases studied. Even

the mesentoderm being added, when the graft happened to remain in the intestinal lumen, degeneration ensued and the mesectoderm failed to become cartilaginous.

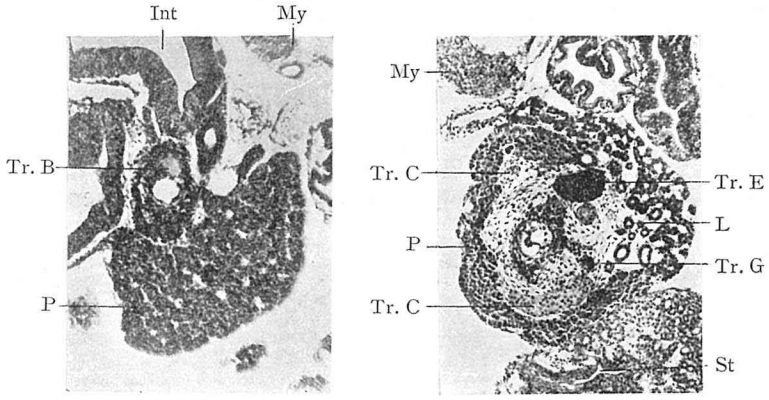

Fig. 7. *a*, no formation of cartilage from the transplanted mesectoderm which happened to lie between the pancreas and the intestinal canal, notwithstanding the development of mesenchyme and pigment cells. Brain tissue is differentiated from the neural cells contained in the graft. IMPJ (19/II-1/III, 1934) *b*, production of cartilage from the mesectoderm transplanted along with the underlying mesentoderm between the liver and the pancreas. IMPI (19/II-1/III, 1934) Int, intestine; My, myotome; L, liver; P, pancreas; St, stomach; Tr. B, transplanted brain; Tr. C, transplanted cartilage; Tr. E, transplanted eye; Tr. G, transplanted ganglion.

In the case of implantation into the yolk mass, grafts were always found in the lateral mesoderm. Though STONE failed to obtain cartilaginous differentiation of the mesectoderm in such position, I have found chondrification distinctly. Fig. 8 is one of the representative cases, where the cartilage is formed attaching even to the intestinal canal which makes an unfavourable condition for chondrification.

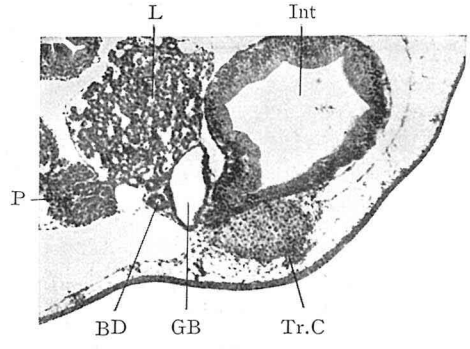

Fig. 8. Cartilage produced from the transplanted mesectoderm in the abdominal wall. AE 7 (16/III-25/III, 1934) BD, bile duct; GB, gall bladder.

c) *Transplantation into the mesentodermal environment*

In this series of experiment, grafts were taken from the

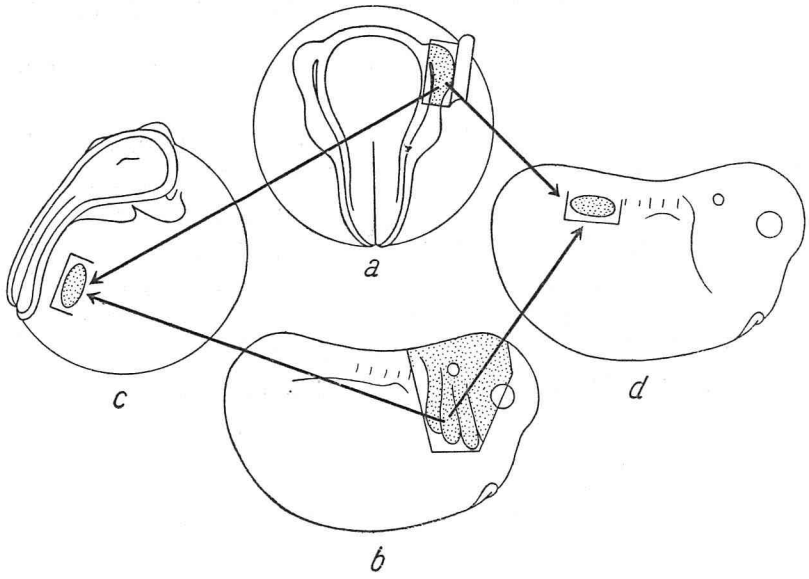

Fig. 9. Schema, showing the transplantation of the mesectoderm into the somatic mesentoderm.

embryos ranging from stages 1 to 7 in development, and were brought into a small hole produced in the somatic mesentoderm or inserted simply under the ectoderm of the body side (fig. 9). The wound was in the former case covered by a piece of the donor's ectoderm, while in the latter it was only pressed by a glass load in order that its margin might close. The results were essentially the same in both cases.

HET 65 (21/III–1/IV, 1934) The mandibular mesectoderm of an embryo in stage 2 was grafted under the skin of the host in a slightly advanced stage. Three hours later the wound healed completely, leaving an elevation in the grafted area. The elevation was gradually flattened and became finally indistinguishable from the general surface. On the eleventh day from the operation the larva was preserved in BOUIN'S mixture and prepared in cross sections.

Histological examinations show that the graft migrates in the ventromedial direction under the myotomes up to the notochord, where it develops into cartilage which lies along the main axis of the body. This mode of migration of the graft in the ventromedial direction is universal in all specimens of this series of experiment, but the reason why it moves in such direction is uncertain. This can be ascribed partly to its own tendency to move inwards and

partly to the cellular movement of the host, especially in the region where the gonad is formed.

The myotomes on the operated side are forced to shrink due to an unusual development of the cartilage under them (fig. 10, *a*). In the specimen under consideration two cartilages are found, one being as long as 890 micra and the other about 80 micra, attaching to the anterior end of the other. Besides cartilage the ganglionic and mesenchymal tissues are also produced from the grafted mesectoderm. The formation of these tissues is more clearly shown in fig. 10, *b*, which belongs to another specimen of the same series of experiment (HET 10, 12/II-23/II, 1934). In this specimen moreover a piece of brain is produced (fig. 10, *b*; Tr. B). This is, however,

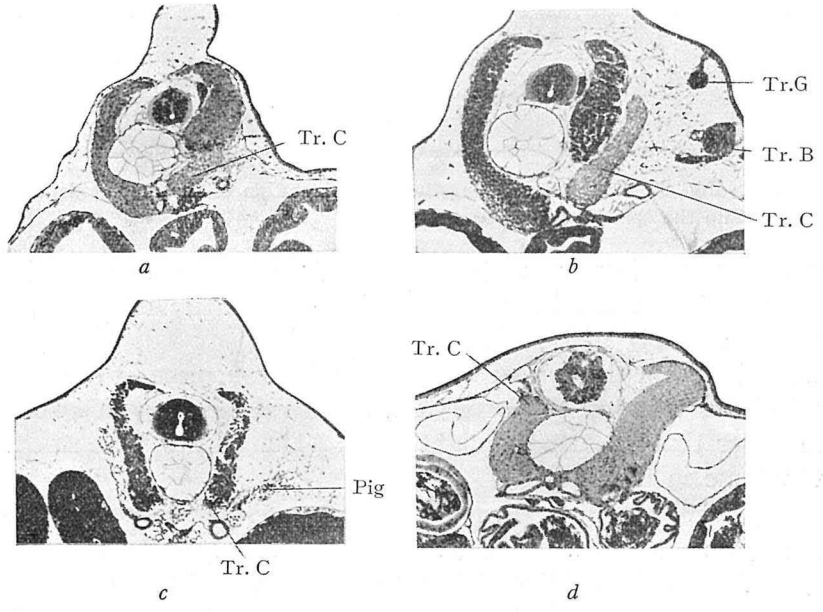

Fig. 10. Formation of cartilage from the transplanted mesectoderm in the somatic mesentoderm. In *a* and *b* the graft was taken from the embryo in stage 1, while in *c* and *d* from the embryo in stage 7. *a*, HET 65 (21/III-1/IV, 1934); *b*, HET 10 (12/II-23/II, 1934); *c*, HET 52 (28/II-8/III, 1934); *d*, TRANS 7 (16/II-2/III, 1935). Pig, pigment.

not referable to the prospective potency of the mesectoderm but perhaps due to the neural tissue contained in the graft.

HET 52 (28/II-8/III, 1934) The massive ventral portion was used as the graft, the method of transplantation being the same as in the preceding case. The transplanted place was slightly mounded, but later became smooth also in this case as in the preceding case.

The specimen was fixed 8 days after the operation. As the result the graft was found to be degenerated for the most part into dark pigments, but a small portion developed to a procartilaginous state. No ganglion was formed in this case. In another specimen—TRANS 7 (16/II–2/III, 1935)—which was preserved 6 more days than the preceding case, chondrification is farther advanced; as clearly shown in fig. 10, *d* a small cartilage is formed lying parallel to the spinal cord. In this case also, no ganglion is produced, and myotomes are small in consequence of their dorsal portion having been removed when the hole, where the graft was brought in, was made previously.

d) *Short conclusion from above results*

From the foregoing experiments, especially implantation into the brain-cavity, we can conclude that the mesectoderm gives rise directly to the cartilage, and that chondrification continues even in the absence of the mesentoderm, though the latter, notwithstanding its different origin, leads the process more beneficially.

The results of experiment vary according to the developmental stage of the graft. When the mesectoderm is taken from an early neurula, cartilage, mesenchyme and ganglion are produced, but in later stages when the downward extention of the mesectoderm takes place, the ganglion alone fails to develop. This is perhaps due to the fact that the potency of ganglionic formation is separated from the original mesectoderm as the latter makes its downward movement along the brain side. This does not speak of the morphological segregation in the cellular group, but means the potential segregation of the branchial mesectoderm.

B) *Extirpation experiments of the cranial mesectoderm*

The lateral side of the head was incised in U-shape in embryos of stages from 3 to 7, and the ectodermal flap was turned dorsally over to make removal of the underlying mesectoderm. After thus removing any group or groups of the mesectoderm, the flap was turned back to heal the wound. In the case of the mandibular group it is absolutely necessary to use younger embryos when the branchial mesectoderm is not yet individualized, because, after this process has started, the mandibular group is closely applied to the mesentoderm, and it becomes very hard to extirpate the mesectoderm alone. As a consequence, the results are unreliable.

In general the part of extirpation and the degree of deficiencies can be discerned superficially. When the mandibular group is

removed, the mouth part shows great malformation on the operated side, resulting in the dislocation of the eye and nostril on that side. If the extirpation is of the hyoid group, a shallow dorsoventral depression is produced in a region ventrocaudal to the eye. The extirpation of the branchial groups is followed by the emaciation of the branchial region of the embryo when the internal gills are formed, the development of the latter being arrested and their supporting cartilages being absent.

The external gills are sometimes affected and sometimes not according to the stage of development when operation is made, i. e., the operation in early stages yields almost no effect, while in later stages, it results in the small and abnormal shape of the organ. For instance, if the first group of the branchial mesectoderm was removed in stage 6 or 7, the first gill was affected and if the second group was removed, malformation of the remaining gills occurred. This fact indicates that the mesectoderm possesses another faculty besides cartilaginous formation, namely it is concerned with the mesenchymal formation of the gills.

Of 144 operated embryos, 121 available specimens were obtained, among which a few cases are selected for the following description.

a) *Extirpation of the mesectoderm on one side*

1) *Removal of all mesectodermal groups*: Extirpation was done in stages 1 to 3 on the right side of the head as shown in fig. 11.

RRM 11 (8/III–17/III, 1934) Operation was performed in stage 2. The external gills were not arrested from development by the operation, but organs such as mouth, eye and nostril were dislocated to the asymmetrical position. On the ninth day after the operation the specimen was fixed and examined in horizontal sections. According to this observation the eye remains not only in displacement, but quite abnormal in structure. It is smaller and less differentiated as compared with that of the other side, namely a narrow space, which passes directly to the brain-cavity without communication of the optic stalk, is still visible between retina and tapetum as a remnant of the original condition of an optic vesicle. The optic nerve can not be detected on the operated side. Further the structural defect of the optic cup checks the development of

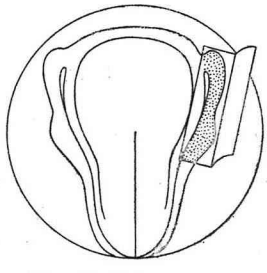

Fig. 11. Schema, showing the area from which the mesectoderm was removed.

the lens, which is small and vesicular without differentiation of fibres. However, the abnormality of the eye would not be a direct result of the operation, but would be secondarily brought about by the lack of cartilages as well as of mesenchyme which should be

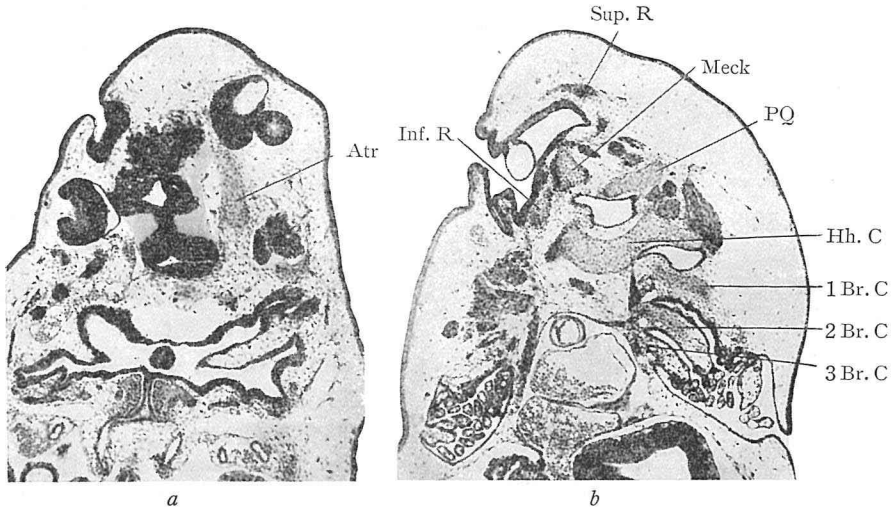

Fig. 12. *a*, frontal section, showing the absence of the anterior trabecular bar due to previous extirpation of the cranial mesectoderm from the right side (left in figure); *b*, the same specimen, 34 sections ventral to *a*, showing also defects of other cartilages on the operated side. RRM 11 (8/III-17/III, 1934) Atr, anterior trabecular bar; 1-3 Br. C, first to third branchial cartilages; Hh. C, hypohyal; Inf. R, infrarostral; Meck, MECKEL's cartilage; PQ, palatoquadrate; Sup. R, suprarostral.

derived from the extirpated mesectoderm. The arrested development of the nose on the operated side may be also traced to the same reason. But by far the greatest deficiency is found in the visceral cartilages. As fig. 12, *b* shows there is no formation of cartilages such as supra- and infrarostral, MECKEL's cartilage, palatoquadrate, hyoid and branchial on the operated side (left in figure). There is further no formation of trabecular bar (fig. 12, *a*). Therefore these cartilages are assumed to be derived from the mesectoderm. The second and third branchials are represented by small pieces, but the fourth is absent. This appearance of branchial cartilages can be ascribed to a technical failure, the extirpation not having been extended far enough posteriorly to the region where the cartilages originate. On the other hand the formation of the auditory capsule is least influenced by this experiment. This fact suggests the organ being of the mesentodermal origin and not of the mesecto-

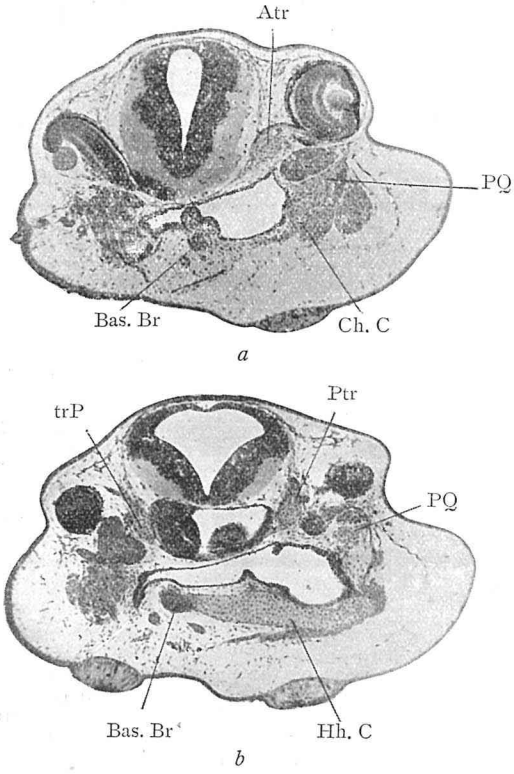

Fig. 13. *a*, cross section through the level of eye, showing the absence of cartilages on the side from which the mesectoderm was previously removed. *b*, same individual, 15 sections posterior to *a*, showing the compensatory extention of hypohyal of the unoperated side, and the absence of mesectodermal cartilages on the operated side. RRM 9 (8/III-17/III, 1934) Bas. Br, basibranchium; Ch. C, ceratohyal; Ptr, posterior trabecular bar.

dermal derivation. This problem will be discussed in another place.

RRM 9 (8/III–17/III, 1934) In this case the specimen was examined in transverse sections perpendicular to the main axis of the body. But owing to the asymmetrical development of the organs in the operated region, especially near the anterior end of the head, we could not detect the same organ at the same level. Moreover the eye of the operated side has a ventral defect (fig. 13, *a*), nevertheless the lens differentiates normally and shows a fibrous structure comparable to that of the other side. Without measurement it can easily be recognized from fig. 13, *a* that the amount of the mesenchyme in the operated region is less than on the intact side. The anterior trabecular bar is absent, but the posterior portion is shown in the other section (fig. 13, *b*) which passes through a level posterior to that of fig. 13, *a*. The palatoquadrate and hypohyal are both absent, and the lack of the latter induces, having perhaps compensatory meaning, the supernormal development of the same cartilage on the other side, resulting in the pushing of the basibranchium beyond the mid line to the operated side. The supra- and infrarostral and MECKEL's cartilages are all absent. The first branchial is also deficient, while the second and third branchials are presented in much reduced form. The fourth branchial cartilage is

again missing. This may be due to an insufficient amount of the mesectodermal cells of the third branchial group from which the fourth one is separated.

The development of the auditory capsule and muscles is normal and not effected by the operation. As a whole the present specimen shows nearly the same degree of malformation as in the preceding case.

Thus the results of the extirpation experiment are quite affirmative to the conclusion drawn from the previous graft experiment, i. e., that the cranial mesectoderm gives rise to the cartilage. But

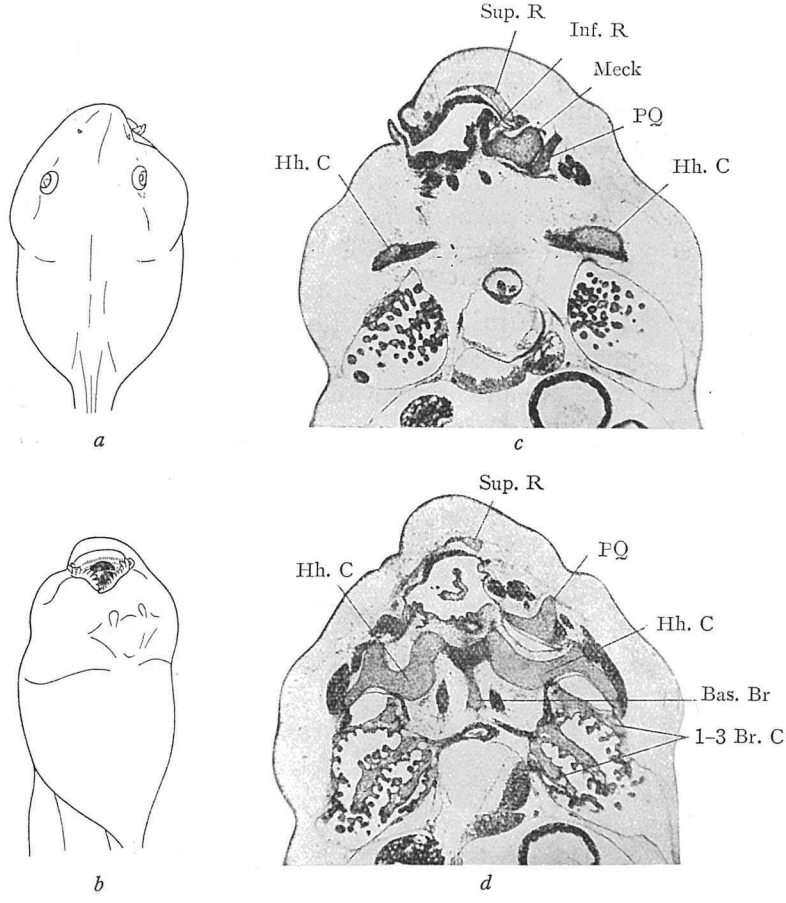

Fig. 14. *a,* dorsal view, showing the defects due to previous extirpation of the mandibular mesectoderm. *b,* ventral view of the same specimen. *c-d,* frontal sections of the same individual, showing the absence of cartilages such as supra- and infrarostral, palatoquadrate and MECKEL's cartilage on the operated side. RMn 12 (20/III-30/III, 1933)

the question is still open as to which cartilage is derived from which group of the mesectoderm. Therefore the next experiment is to remove any desired group of the mesectoderm only.

2) *Removal of the mandibular group*: Of 13 operated embryos in this series of experiments, 9 remained useful. Since, however, the degree of malformation is nearly the same in all specimens, one RMn 12 (20/III–30/III, 1933) is selected for the description. Operation was made on the embryo in stage 4. Deficiencies are distinct; especially the mouth is shifted to one side. It can be seen even in the dorsal view, a deep depression being produced in front of the eye of the operated side as shown in fig. 14, *a* and *b*. Nevertheless this specimen, while living, could take food.

Fig. 14, *c* and *d* represent two sections passing through the cranial region of the above specimen. In these figures hyoid and other posterior cartilages are well developed on both sides, while supra- and infrarostral, palatoquadrate and MECKEL's cartilage are produced only on one side. This fact shows that the cartilages of the latter group are derivatives of the mandibular group of the mesectoderm.

3) *Removal of the hyoid group*: Removal of this group involves two important meanings, one being to show what cartilages are deficient after this treatment and the other being to verify the results obtained in the preceding experiment, that is to say, this

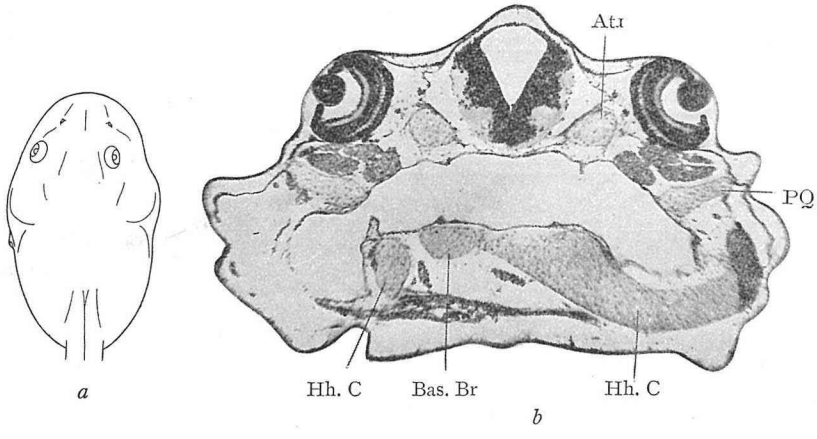

Fig. 15. *a*, dorsal view, showing the slight depression below the right eye due to previous extirpation of the hyoid mesectoderm. *b*, cross section of the same specimen through the level of eye, showing the defect of the hyoid cartilages alone of the operated side and the remarkable extention of the same cartilage of the opposite side. RHy 19 (21/III–7/IV, 1933)

extirpation does not effect the development of the cartilages mentioned above as mandibular derivatives.

Twenty embryos were operated for this purpose in stage 7 and all survived to the end of experimentation.

In one example RHy 19 (21/III–7/IV, 1933) a slight depression was at first produced in the region ventrocaudal to the eye, but later became obscure (fig. 15, *a*).

Histological examination of this specimen shows that the cartilages of mandibular derivatives are not effected and their development is normal like those on the other side. Defects are however noticed in the hyoid cartilage. As shown in fig. 15, *b*, hypohyal is very small and does not meet the partner of the opposite side, notwithstanding the compensatory extention of the latter beyond the median plane into the field of the operated side. Such enlargement of cartilage is common to all specimens where the development of cartilage is failed on the operated side, the basibranchium being accordingly forced to take an exceedingly shifted position. But this cartilage is not so small in spite of its one-sided origin. In normal development it is of a double origin, the material being contributed from each side.

4) *Removal of the branchial groups*: Operations were carried out on the embryos in stage 7 when two pairs of branchial mesectoderm, anterior and posterior, were established. The anterior and posterior groups were separately removed to see which cartilage or cartilages are respectively derived from each.

Nine individuals were obtained in the lot of anterior removal, of which the specimen RABr 1 (14/III–21/III, 1934) is selected as an example. The first gill is smaller than the normal on the opposite side in agreement with the findings of STONE (l. c.) in American species.

In sections as represented by fig.16, *b–d* there is no trace of the first branchial cartilage. The fact would show that the anterior group of the branchial mesectoderm gives rise to the first branchial cartilage. Owing to the absence of this cartilage the second one, i. e., ceratobranchial elongates to the level of basibranchium to form the hypobranchial plate, though the overgrown part is slender as is shown in fig. 16, *c*. In normal development, the main part of the hypobranchial plate is an extention of the posterior border of the first hypobranchial and the second takes part in its formation. The above mentioned overgrowth of the second

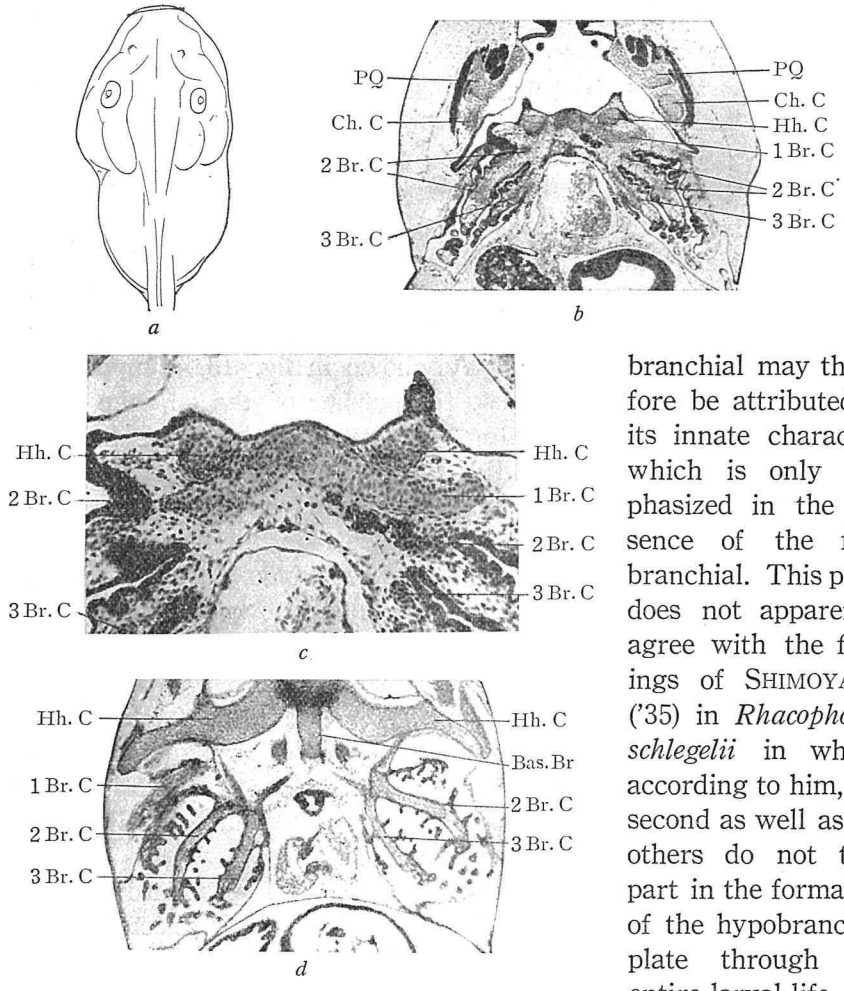

Fig. 16. *a,* dorsal view, showing deficiencies of the branchial region due to previous extirpation of the anterior group of branchial mesectoderm. *d,* frontal section of the same specimen, showing the absence of the first branchial cartilage on the operated side. RABr 27 (30/I-20/II, 1935) *b,* frontal section, showing the lack of the first branchial cartilage; *c,* 2 sections posterior to *b,* showing the compensatory extention of the second branchial due to absence of the first. RABr 1 (14/III-21/III, 1934)

branchial may therefore be attributed to its innate character, which is only emphasized in the absence of the first branchial. This point does not apparently agree with the findings of SHIMOYAMA ('35) in *Rhacophorus schlegelii* in which, according to him, the second as well as the others do not take part in the formation of the hypobranchial plate through the entire larval life, but they are only connected to the proximal portion of the first ceratobranchial by his so-called *commissura proximalis.*

As for the posterior group of the branchial mesectoderm, out of 26 available cases, the specimen recorded as RPBr 2-22 (13/III-20/III, 1934) is selected as the type of description. The posterior group and the mesectoderm situated still

further behind, sticking on the brain-side, was removed as thoroughly as possible. As the result the development of the second and third external gills was suppressed, being provided only with a few short filaments against the normal development of the first gill. This contrast of gill development between the first and others is particularly important as an affirmation of the preceding experiment in which the anterior group of the branchial mesectoderm was

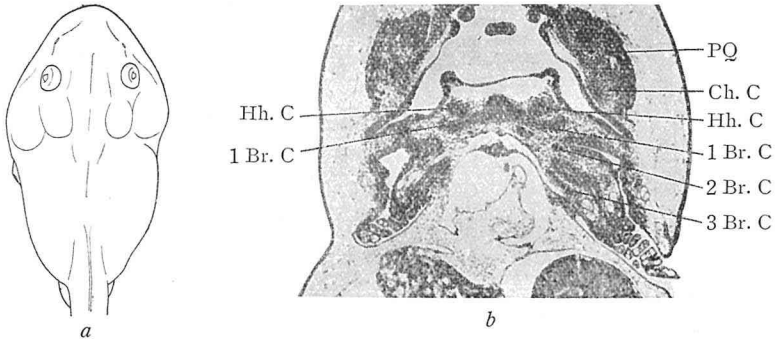

Fig. 17. *a*, dorsal view, showing deficiencies in the branchial region due to previous extirpation of the posterior group of the branchial mesectoderm. *b*, frontal section of the same specimen, showing the absence of branchial cartilages, except the first. RPBr 2-22 (13/III-20/III, 1934)

removed resulting in no effect on the formation of the second and third gills. The corresponding second and third internal gills were also arrested in development on the operated side, and the specimen appeared emaciate in this region (fig. 17, *a*).

Internal structure of the branchial region is shown in fig. 17, *b*; the development of the anterior portion of the visceral cartilages, including the first branchial, is observed to be quite symmetrical on each side. The second and third branchials are, however, presented only on one side and the fourth is entirely absent on both sides, though in other sections this cartilage is found on one side.

Further, in a few cases, a small cartilage is found at the second branchial portion of the operated side, but this may be due to the failure of the complete extirpation of the tissue in question. But in any case the experiment demonstrates that the branchial cartilages, except the first, are derived from the posterior group of the branchial mesectoderm.

b) *Extirpation of the mesectoderm on both sides*

This experiment, which excludes the mesectoderm from the hyoid and branchial regions, was performed to see the effect of the

lack of the mesectoderm upon the development of the second basibranchium as well as of the auditory capsule. But for the sake of convenience the two organs will be considered separately.

1) *The second basibranchium*: In the normal development of the basibranchium the caudal portion is found to contain many yolk granules even after the basal portion has exhausted them, suggesting its double origin, i. e., according to STONE the basal portion is derived from the mesectoderm and the caudal portion from the mesentoderm. Particularly to verify this point 33 embryos were operated in stage 7 by removing the hyoid group from both sides of the head. The defect is more remarkable than in one-sided extirpation, especially in the ventral aspect; the head being narrower in width and shorter in length in comparison with the normal specimen (fig. 18, *a* and *b*). The internal gills are more or less displaced towards the anterior region.

RBHy 36 (12/III–19/III, 1935) This specimen was fixed when the mesectodermal cartilages were still in procartilaginous stage, but sufficient for the

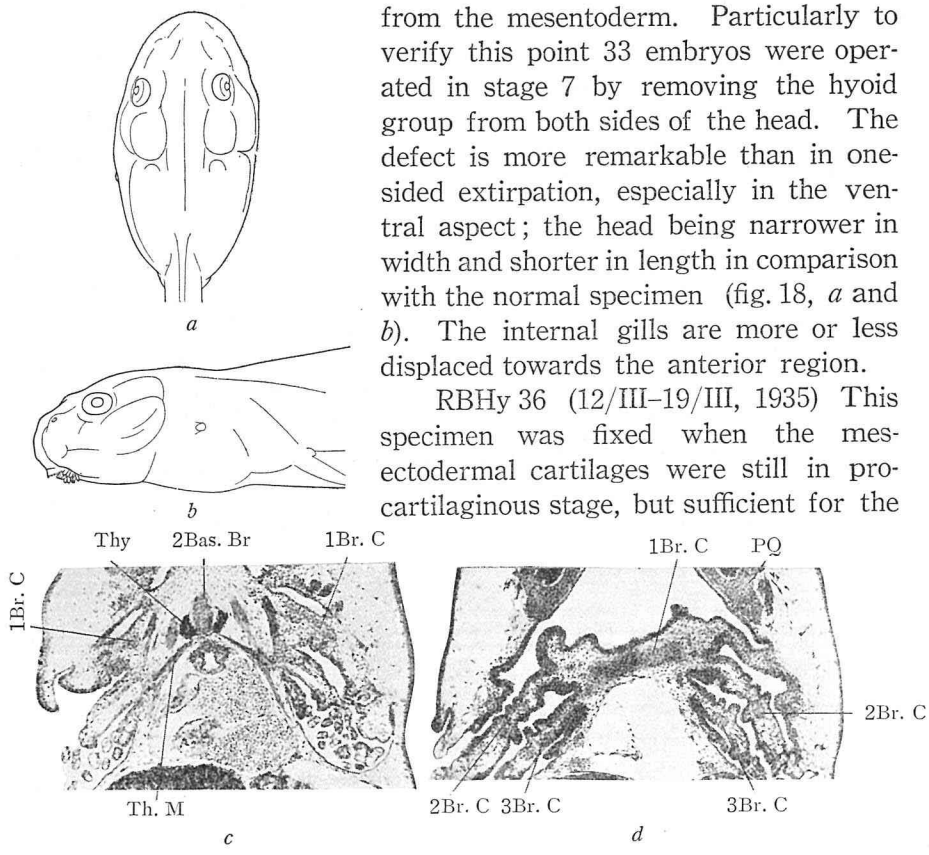

Fig. 18. *a*, dorsal view, showing slender appearance of the head due to previous extirpation of the hyoid mesectoderm of both sides. *b*, lateral view of the same specimen. RBHy 7 (5/II–23/II, 1935) *c*, frontal section, showing the formation of the second basibranchium in the absence of the first as well as other cartilages derived from the hyoid mesectoderm. *d*, 11 sections dorsal to *c*, showing also the absence of the hyoid cartilages. RBHy 36 (12/III–19/III, 1935) Th. M, thoracicopharindeus muscle; Thy, thyroid.

study of the development of the hyoid group. The photomicrographs of the section through the level of the branchial portion are re-

presented in fig. 18. The palatoquadrate is quite normal but the first branchial cartilages are somewhat modified, they meet each other directly due to the absence of the basibranchium which interlocates between them (fig. 18, *d*). However, in front of the heart a small piece of cartilage is present, to which the thoracicopharindeus muscle and thyroid rudiment attach on either side (fig. 18, *c*). Judging from its position, the mode of muscular attachment and the presence of thyroid, this cartilaginous piece is regarded as an equivalent of the second basibranchium. Therefore the basibranchium which is missing in the specimen represents the part derived from the mesectoderm, namely the first basibranchium. A similar defect of the internal structures is more clearly shown in fig. 19 which is prepared from another specimen (RBHy 21) preserved

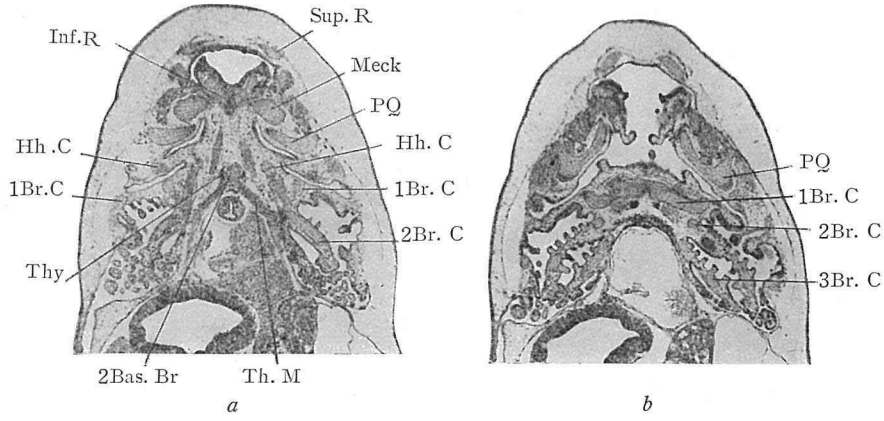

Fig. 19. Similar to preceding, showing the development of the second basibranchium in spite of the previous extirpation of the hyoid mesectoderm on both sides. RBHy 21 (12/III-22/III, 1935)

3 days later. In this time cartilaginous differentiation advances and there are no more yolk granules in the mesectodermal cartilages, while abundant in those of the mesentodermal origin. The fact that a small piece of cartilage in front of the heart still contains many yolk granules would speak of its origin as the mesentoderm. The attachment of the thoracicopharindeus muscle and the presence of thyroid rudiment further prove the cartilage as the second basibranchium. In this case very small ceratohyals are produced in spite of the hyoid groups of the mesectoderm being removed, but the first basibranchium is absent. The development of the other visceral certilages is entirely normal. However, that other cartilages beside the hyoid are present near the second basi-

branchium makes doubt anew on the origin of this latter cartilage, though it contains yolk granules in contrast to the mesectodermal ones. To solve the question it is desirable to take off all mesectodermal cartilages in this region.

Both hyoid and branchial mesectoderms were next removed on both sides of the embryo in stage 7. Even after this extirpation if we can get the development of the second basibranchium, this cartilage is really proved to be of the mesentodermal origin. Ten specimens were operated for this purpose and all were studied carefully by serial sections.

RBHyBr 6-2 (31/III-13/IV, 1936) This representative specimen was operated in stage 7. It showed a peculiar shape with the head particularly reduced in size due to the defect in the branchial region. Since, however, the defect was of the same degree on both sides, the tadpole presented a bilateral symmetry as a whole.

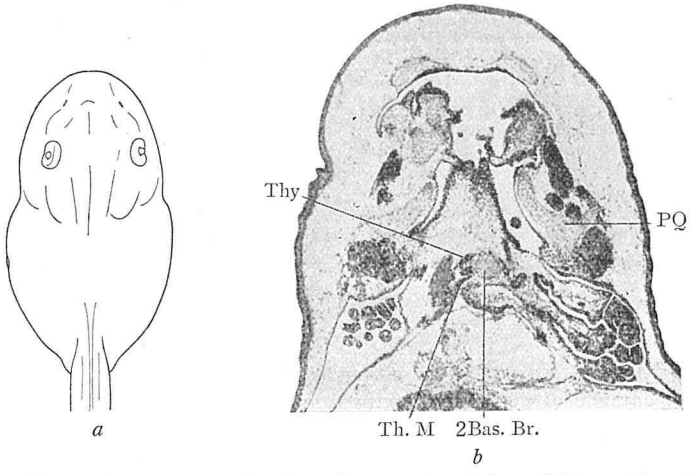

Fig. 20. *a*, dorsal view, showing the emaciated branchial portion due to previous extirpation of both hyoid and branchial mesectoderm from both sides. *b*, frontal section of the same specimen, showing the mesentodermal origin of the second basibranchium. RBHyBr 6-2 (31/III-13/IV, 1936)

A study of sections reveals that cartilages to be derived from hyoid and branchial groups of the mesectoderm are all absent in the branchial region of this specimen. This fact shows clearly the successful removal of the mesectoderm. Nevertheless the second basibranchium alone is present in front of the heart, being accompanied by the thoracicopharindeus muscle and thyroid rudiment. Therefore we can say that the second basibranchium is the derivative of the mesentoderm which is not effected by the operation.

Thus that the basibranchium is, in its genesis, composed of two parts of different origin, i. e., mesectodermal and mesentodermal, is substantiated in *Rana japonica* in agreement with the findings of STONE in the frog *Rana palustris.*

2) *The auditory capsule*: Now turning to the similar problem of the origin of the auditory capsule, there are two opinions; one holds to its mesectodermal origin, while the other maintains its mesentodermal derivation. In former papers I have reported that

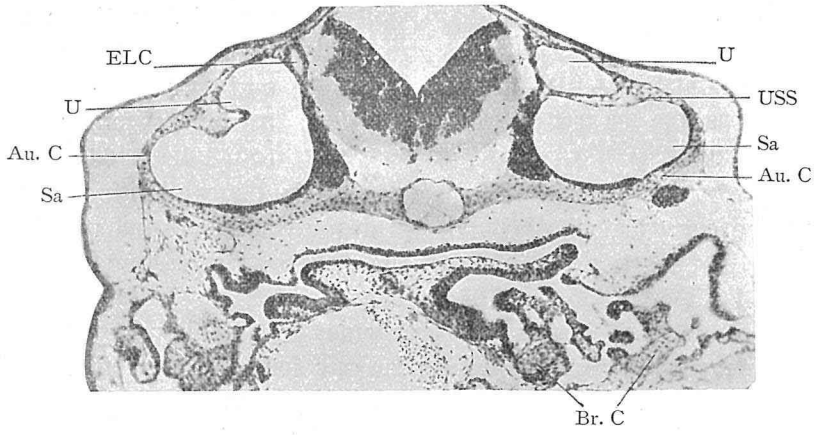

Fig. 21. Cross section, showing the mesentodermal origin of the auditory capsule; notice no structural difference of the organ on both sides in spite of previous extirpation of the mesectoderm from the otic region of one side (left in figure). RHyBr 2 (12/III-22/III, 1935) ELC, endolymphatic canal; Sa, saccule; U, utricle; USS, utriculo-saccular septum.

the latter opinion is reliable in Japanese species, *Triturus pyrrhogaster* and *Rana japonica.* The proof is obtained by experiments on the cranial mesectoderm both by transplantation and by extirpation. Its removal did not affect the capsular formation, and its transplantation along with the auditory vesicle failed to form the capsule. The capsule was only produced when the auditory vesicle was transplanted along with the neighbouring mesentoderm. Fig. 21 represents a section of the specimen, in which the mesectoderm was previously removed on one side of the branchial region. In this figure we find no structural differences between the capsules on both sides, notwithstanding the absence of branchial cartilages on the operated side (right in figure). This developmental contrast between the auditory capsule and the branchial cartilages proves that one is derived from the mesectoderm and the other from the mesentoderm, i. e., the capsule is formed from the mesentoderm.

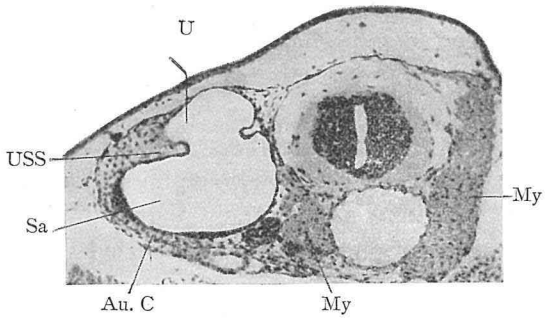

Fig. 22. Cross section, showing the auditory capsule differentiated from the mesentoderm transplanted along with the auditory placode. AuM 4 (19/III-30/III, 1935)

Fig. 22 represents the case where the auditory vesicle is transplanted along with the mesentoderm and produces the capsule. The development of this heterotopic ear is quite normal and well differentiates into its two parts; saccule and utricle, with the utriculo-saccular septum between them. The sensory area of the saccular portion is also well differentiated. Thus the ear as a whole is normal despite its abnormal position.

Further support is given by the experiment of removing the mesectoderm on both sides of the head; in spite of the operation the development of both auditory capsules is normal and not effected in any degree. Therefore we repeat our former statement that the auditory capsule is derived from the cranial mesentoderm.

c) *Short conclusion from above results*

To sum up the results so far obtained, the mandibular group of the mesectoderm gives rise to cartilages such as supra- and infrarostral, MECKEL's cartilage, palatoquadrate as well as the anterior trabecular bar. The hyoid group becomes hyoid and the first basibranchium. The anterior group of the branchial mesectoderm (in stage 7) produces the first branchial and hypobranchial plate as well, and the posterior group provides other branchials. The second basibranchium and the auditory capsule are derived exclusively from the cranial mesentoderm.

C) *Determination problem*

As demonstrated in the above experiments, the cranial mesectoderm is particularly concerned with the formation of the visceral cartilages, and it is necessary to explain when the tissue aquires this faculty. As this problem has not yet been studied in any species of anurans, the next investigation is carried out on this point. The method of transplantation was applied for this purpose, using, as donors, younger embryos than those in the preceding experiment.

Since the term "determination" as employed by different authors

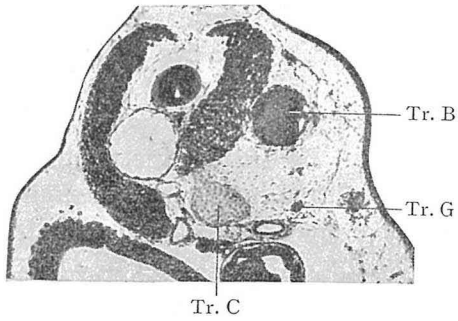

Fig. 23. Cross section, showing the differentiation of cartilage and ganglion from the transplanted mesectoderm; graft being taken from the embryo a little earlier than stage 1. HET 7 (12/II-23/II, 1934)

varies, it is necessary first of all to give here the definition adopted in the present paper. When a graft, after transplanting into a heterotopic position, yields always negative results as to the desired character, it is designated as undetermined; if the result is sometimes positive and sometimes negative, it is assumed the character is in labile determination, and finally if the positive result only is obtained, we say the determination is irrevocable so far as the experimental character is concerned. In short, when the prospective fate of the graft is greater than the assimilative influence of the environments, the tissue is said to be determined.

In the first experiment the grafting mesectoderm was taken from an early neurula in which the medullary plate was faintly defined, and the tissue was transplanted into the body side. Out of 12 cases all produced cartilages in such a heterotopic position (fig. 23).

In another experiment, one side of the anterior portion of the medullary plate was reversed to the opposite position involving the mesectoderm so that the latter became situated between the neural tissues. Out of 10 cases, 7 brought the positive results of cartilage formation and the rest died before giving any useful results. In general the mesectoderm did not remain in the neural tissue, but sank deeply into the mesenchyme near the chorda where it differentiated into cartilages. The reversed half of the medullary plate sometimes fused in various degrees with the intact side, but in other cases produced an independent tube. In fig. 24 (ROT 7, 20/III-1/IV, 1934) two portions of the operated neural tube are shown, fused in *a* and separated in *b* which is taken 17 sections posterior to the other. A cartilaginous piece can be seen near the chorda. Such locality of the cartilage might be mistaken for the initial vertebra, but it is certainly not, because the differentiation of the latter is not yet so advanced as to present a cartilaginous structure as in the piece in question.

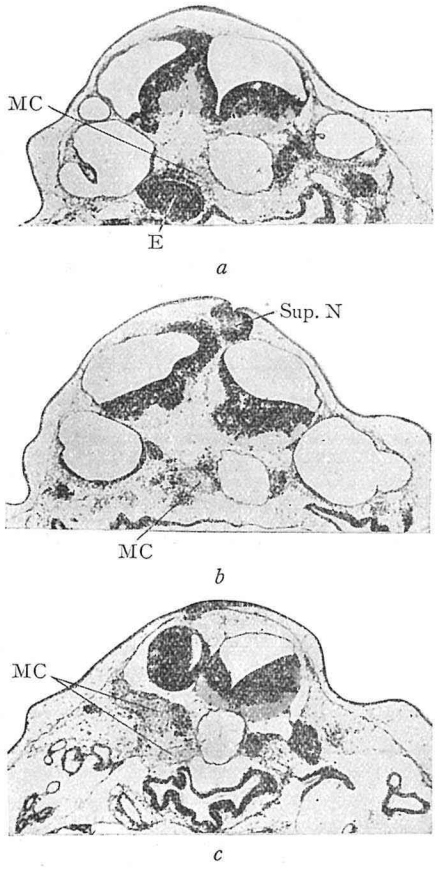

Fig. 24. Cross section, showing the differentiation of cartilage, eye, brain and nose after reversion of the cranial mesectoderm including some part of the neural tissue so that the former appears to be placed between neural tissues. *a* and *b*, ROT 7 (20/III-1/IV, 1934); *c*, ROT 8 (20/III-1/IV, 1934) MC, mesectodermal cartilage; Sup. N, supernumerary nose.

Of the formations besides the cartilage an eye is produced, with well differentiated retina and tapetum, but without the lens. By far the most interesting formation is the appearance of a supernumerary nose at the position dorsomedian to the ear. Whether it is induced by the inverted fore-brain or whether the nasal tissue has been introduced in this position is a question.

The two experiments above described prove that the mesectoderm creates a cartilaginous formation by the time the blastopore is reduced to a slit-like depression and the medullary plate is faintly defined.

Next the same experiment was repeated in a much younger stage, i. e., on the gastrulae with a yolk-plug of middle size. (As the recipient embryos were a little older: late gastrulae were sometimes employed.) It is, however, very difficult in these young embryos to distinguish the presumptive mesectoderm from other tissues on account of the obscurity of its boundary. Therefore, after the graft was removed, the wound was covered either by a piece taken from another embryo of somewhat different colour or even by the tissue of *Hynobius*. Sometimes the wound was left uncovered, and the donor embryos were reared up to the stage when they showed the neural folds in order to make sure of the position of the graft.

Furthermore, in such young stages it is almost impossible to prevent the graft from containing other presumptive tissues such as neural or epidermal one.

Fourteen embryos were operated, but only 4 remained useful; these were studied in sections. In all cases, mesenchymal, ganglionic and nervous tissues were produced, but there was no sign of cartilaginous formation. In the preceding experiment, i. e., transplantation of the mesectoderm into the endodermal environment, mesenchyme and ganglion were produced in spite of no formation of the cartilage. These facts seem to prove that those tissues, such as ganglion and mesenchyme, may be determined earlier than the cartilage in the formation of the mesectoderm.

In another set of experiments, gastrulae in a slightly advanced stage were used. Six specimens were used for section, among which 4 showed the differentiation of cartilage, while others failed

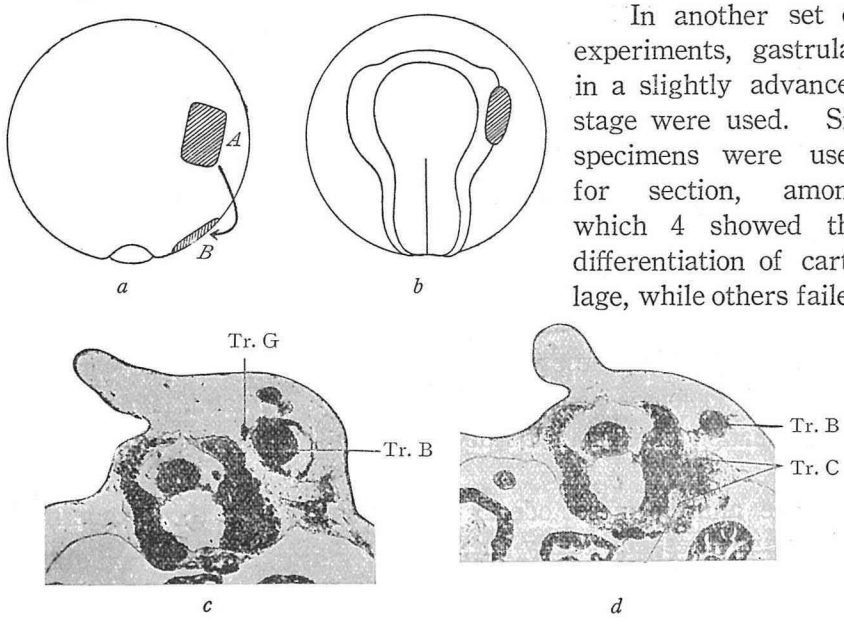

Fig. 25. *a*, protocol sketch, showing the method of operation; graft taken from the area A and transplanted to the area B of another individual. *b*, camera-drawing of the donor 4 hours later than *a*, showing the position from which the graft was taken. *c* and *d*, cross sections of the same specimen, showing the formation of cartilage, ganglion and brain from the graft. DET 31 (19/III–1/IV, 1934)

to present it. Therefore, so far as my definition is concerned, the mesectoderm in this stage of development can be regarded as transitional with respect to the cartilage-formation. The determination becomes definite 2 or 3 hours later when the yolk-plug is completely withdrawn.

DET 31 (19/III–1/IV, 1934) Donor and recipient were in the same stage, the small yolk-plug being elliptical rather than circular. The graft was transplanted into the region anterolateral to the

blastopore of the host without underlying tissues (fig. 25, *a*). The wound of the donor was left uncovered and became smaller when the neural folds appeared 4 hours later; it was located on the broad portion of the fold. Thus the graft was shown to be removed correctly from the mesectodermal position (fig. 25, *b*). Thirteen days after the operation the host was fixed and studied in sections. Two pieces of cartilage were found, one being small and located outside the myotome and the other large and situated beneath the myotome (fig. 25, *d*). Of other tissues, ganglion, brain, mesenchyme and pigment cells were formed (fig. 25, *c*).

IV. General considerations

The medullary plate, when first defined, consists of three kinds of tissues:—neural, mesentodermal and mesectodermal, of which the first occupies the large frontal portion and gives rise to the neural tube. The second takes the posterior portion of the plate extending to a certain distance in front of the blastopore and is concerned with the formation of the somatic mesentoderm of the tail (BIJTEL, '31 and '36). The mesectoderm, to which the present investigation is related, is located on each side of the plate and takes part in the formation of various tissues such as ganglion, mesenchyme and cartilage.

The anuran mesectoderm closely resembles the ectoderm in colour and presents nearly the same cytoplasmic structure, but differs even in an early stage of development from both tissues, neural and epidermal, in the shape and arrangement of its cells. The mesectoderm elevates as the primary neural fold, then its cranial portion commences the characteristic downward movements over the mesentoderm of visceral arches, when the secondary (inner) neural fold begins to appear. By the time the latter is closed into the neural tube, migration of the mesectoderm extends considerably; it does not relate to the formation of the neural tube at all. In urodeles the mesectoderm closely resembles the neural tissue in shape and arrangement of its cells, being closely related to the latter, and at first incorporated in the neural tube at the dorsal portion. The mesectoderm becomes detached later from the tube and extends ventrally as a sheet of cells (LANDACRE, '21, STONE, '22, '26 and RAVEN, '31).

Those authors who worked on the development of the cranial

skeleton have already been well aware of the presence of two kind cells which relate to it, but as to the origin of the mesectoderm, opinions vary among them, some ascribing it to the ectoderm of the head, while others maintain it belongs to the neural crest. Still others consider that the mesectoderm is derived partly from the lateral side of the head and partly from the neural crest. The dispute is now quite settled and the crest origin of the mesectoderm is generally admitted. In the former paper I ('33) have traced the crest cells of the cranial portion by means of vital staining to the anlage plan of the visceral cartilages. In the present paper the point that the mesectoderm really gives rise to cartilage is demonstrated by transplanting the tissue into the brain-cavity (cf. p. 320). Transplantation to other places supplies further support favourable to the conclusion that the mesectoderm is directly related to the formation of cartilage. But it should be mentioned that the transplanted mesectoderm differentiates more normally at any time in the presence of mesentoderm. Even in the abdominal wall chondrification of the mesectoderm proceeds quite normally as is presented in fig. 8. The chondrification is more or less checked in the ectodermal environment, especially when the mesectoderm of *Rana japonica* was brought into the brain-cavity of *Hynobius nebulosus*. In the latter combination the grafts were all degenerated and there was no sign of cartilaginous differentiation in any case, but if the graft was placed in the mesenchyme outside the brain, cartilage was formed (fig. 5). Therefore, it can be said that the mesectoderm differentiates even in the xenoplastic mesentoderm. In a similar xenoplastic combination between *Rana fusca* and *Triton taeniatus* RAVEN ('33) seems to have obtained the same result. (Refer to his figs. 2–5.) In an endodermal environment, the cartilaginous differentiation of the mesectoderm is practically inhibited (fig. 7, *a*), though it is not quite conclusive on account of the smaller number of experimental cases. In *Rana palustris* STONE ('29) describes the same phenomenon; when the mesectoderm was placed in the endodermal material, there were no signs of cartilaginous differentiation. He also states that there was no formation of cartilage in the abdominal wall. But in the present species the mesectoderm does differentiate into cartilage in the abdominal wall, and this point alone does not agree with STONE's result. In urodele *Amblystoma mexicanum* RAVEN ('35) obtains only in one case a very small cartilage in the pancreas. In my experience, chondrification

is possible only when the mesectoderm is transplanted along with the neighbouring mesentoderm even in the endodermal environments such as between the pancreas and the liver (fig. 7, *b*). In the intestinal canal the graft fails to develop and degenerates in time even when accompanied with the mesentoderm.

Then, when is the mesectoderm destined to give rise to carlilage? According to my experience the determination takes place in *Rana japonica* in the course of the late gastrulation after the stage when the yolk-plug reaches middle size, and becomes definite when the yolk-plug is completely withdrawn. RAVEN ('35) also states that the mesectoderm of *Amblystoma mexicanum* is determined irrevocably when the gastrula with a small yolk-plug advances to a neurula. Therefore, the mesectoderm seems to be destined to give rise to cartilage in nearly the same stage in both anura and urodele, at least so far as the above species are concerned.

In urodele the mesectoderm extends partly by self-proliferation and partly by the continuous supply of cells from the dorsal part of the brain, so that to remove the tissue completely it is necessary to cut off the dorsal part of the brain. In anura the mesectoderm is detached from the original seat in the early stage of development, and the downward extention is simply accomplished by self-proliferation. This makes the complete removal of the tissue easier than in urodele, and the reliability of the results of the removal experiment is accordingly greater. When a desired group of the mesectoderm is extirpated, regeneration from other groups does not as a rule occur, and this is so especially when the operation is done for the mandibular or hyoid group. Sometimes an exceptional case occurs after the removal of the posterior branchial group, when the second branchial cartilage is sometimes formed by regeneration from the anterior branchial group. The removal of the mesectoderm results always in cartilaginous defects corresponding to the group of extirpation; the results of the present experiment on Japanese frogs are well in accord with those obtained in *Rana palutris* and *Amblystoma* by STONE and by RAVEN.

In connection with the problem of the mesectoderm the auditory capsule has been much discussed with respect to its mesectodermal or mesentodermal origin. I am of the opinion that it is a derivative of the mesentoderm as already reported in my previous papers ('35, '36). This view is based on the facts that the development of the auditory capsule was never influenced by the removal of the

cranial mesectoderm (fig. 21) and that the transplantation of the mesectoderm along with the auditory placode or vesicle never resulted in the capsular formation, while, if surrounding mesentoderm was added to it, the cartilaginous capsule was invariably produced regardless of the place of transplantation (fig. 22). In cases where the mesectoderm alone was added to the grafted placode, only a cartilaginous piece or pieces were produced near the vesicle. That RÖHLICH ('29) maintains its mesectodermal origin seems to be based on such a fragmental cartilage obtained in a similar experiment on *Amblystoma sp.* STONE ('22) has already pointed out in *Amblystoma punctatum* that in spite of the complete removal of the mesectoderm in the otic region the development of the auditory capsule is still normal. However, recently REISINGER ('33) proposes again the mesectodermal origin of the capsule in *Rana obstetricans.* In this case the cartilage takes a capsular form, but its origin seems doubtful. I am rather inclined to regard, if analogy from the results of the present frog is allowed, REISINGER's capsule was formed by regeneration from a very small amount of the mesentoderm which he had incidentally carried along with the graft. I know cases where most of the mesentodermal cells were intentionally removed from the transplanted placode, and yet the capsular formation took place quite normally (fig. 22). I believe that the cartilaginous capsule is derived from the cranial mesentoderm. Furthermore, the auditory capsule and the cranial mesentoderm are in an action-reaction system as the eye-cup and the ectoderm in the lens formation. The inducing power of the auditory vesicle is not specific; the anuran vesicle is sufficient to induce the urodele capsule (LEWIS, '07, HOLTFRETER, '35 and ICHIKAWA, '36). Reciprocally the urodele vesicle can also induce the anuran capsule, but this part of my statement is not conclusive, because there are still doubtful parts in my experiments. On the other hand substitution of the auditory vesicle by other sense organs such as eye-cup and nasal sac or by inert materials such as celloidin- and paraffin-blocks resulted in no capsular formation. The attempts to determine the stage when the presumptive mesentoderm of the auditory capsule aquires the ability of self-differentiation were all unsuccessful, partly due to the technical difficulty of removing the vesicle alone without injuring the subjucent mesentoderm.

The possibility of capsular formation seems to be rather restricted to a narrow area of the head (KAAN, '30, YNTEMA, '33),

and the somatic or lateral mesentoderm did not react even to an introduction of the vesicle in this area (ICHIKAWA, '36).

Also from the present investigation it is easily accepted that another important function of the mesectoderm is the production of the mesenchyme; extirpation experiments show clearly less abundance of the tissue on the operated side (figs. 12 and 13), though an accurate quantity can not be claimed simply by a comparison of both sides, due to the dislocation of the symmetrical organs. In the transplantation experiment the mesenchyme is distinctly abundant in the transplanted region (figs. 8 and 10), but how much of the tissue is derived from the transplant and how much is original can not be assumed also in this case. The mesenchymal rôle of the mesectoderm is more clearly shown in the formation of gills, especially when the extirpation is done in later stages of development (RABr 1 and RPBr 2–22). The development of gills is always arrested, the fact presenting clear agreement with the findings of STONE and RAVEN. But such an arrested development does not occur in the early stage (RRM 11). The differences in the results of the experiments according to the different stages may be accounted thus:—When the mesectoderm is removed in an early stage, the proliferation of the mesentodermal cells rapidly takes the place of the extirpated mesectodermal mesenchyme, while in a later stage this compensatory overgrowth of the mesentoderm can not take place, because the formation of gills is close at hand. STONE ('26) has already pointed out that "the mesentoderm can take over the rôle of the mesectoderm in this common function (formation of mesenchyme) when the latter is absent." (p. 128)

One more important faculty of the cranial mesectoderm is the formation of the ganglia. Early investigators on the cranial ganglia put much value on the mesectoderm and considered it as the sole source of their origin. But KUPFFER ('95) has pointed out in *Petromyzon* that the lateral ectoderm is also involved in the formation of the ganglia. In the urodele *Plethodon glutinosus* LANDACRE ('21) observed that the neural crest gives rise to both cutaneous and visceral portions of the ganglia of V, VII, IX and X, and that the special visceral portions of these ganglia as well as the other ganglia and lateral line organs are derived from the placodal cells detached from the lateral side of the head. STONE ('22) first took up the problem from the experimental side using *Amblystoma punctatum* as material and reached the conclusion that all of the general

cutaneous as well as special visceral components of the cranial ganglia are entirely derived from epidermal placodes and only visceral components of V, VII, IX and X ganglia originate from the mesectoderm. Therefore, these results do not quite agree with those of LANDACRE, especially in the point that the general cutaneous system is placodal in nature and not mesectodermal in origin. KNOUFF ('27) described also the origin of the cranial ganglia in *Rana pipiens*, with some differences from the findings of STONE in minor points.

In the present frog the transplanted mesectoderm sometimes produces a ganglion even in a heterotopic position. Although it is not certain from this experiment alone whether this faculty is at first distributed over the entire mesectoderm or whether it is restricted to some parts of it, the ganglionic potency is lost when the mesectoderm passes at the level where the ganglia are laid down (HET 52 and TRANS 7). According to LANDACRE ('21) the ganglionic portion of the mesectoderm becomes grouped *in situ* into 4 conspicuous masses, one mandibular, one hyoid and the other two branchial in level, and in due course they give rise to the ganglia of V, VII, IX and X respectively.

The problem next to be considered is the origin and genesis of the basibranchium. STONE ('22, '26 and '29) has formulated the double origin of this cartilage, i. e., the large proximal mesectodermal portion and the small distal mesentodermal portion. In *Rana japonica* the double structure of the cartilage can easily be detected by the differences in the cytoplasmic structure, if the larva in a suitable stage is investigated. The double nature of the cartilage can be proved experimentally by removing the hyoid and branchial mesectoderm simultaneously on both sides of the head, resulting in the formation of the second basibranchium alone in front of the heart (RBHyBr 6-2). That this portion of basibranchium is due to self-differentiation, STONE ('32) has proved in an ingenious way by transplanting the mesentoderm below the branchial region before the descent of the mesectoderm to this portion, and thus obtaining a cartilage in front of a heterotopic heart in the absence of other cartilages.

In conclusion, we must consider one more problem ; i. e., whether the second branchial cartilage is involved in the formation of the hypobranchial plate. According to SHIMOYAMA ('33) it is related in *Bufo vulgaris japonicus*, and not related in *Rhacophorus schlegelii*

('35). In the present frog I have removed the branchial mesectoderm and tried to prove its incorporation into the hypobranchial plate. In these cases where the anterior group was operated the hypobranchial plate failed to develop, and the position was substituted by an unusual elongation of the second ceratobranchial to the basibranchium (fig. 16, *c*). In those cases where the posterior group of the branchial mesectoderm was extirpated the plate of the operated side was always narrower in its posterior extention than that of the other side. The other branchial cartilages are not concerned in the formation of the hypobranchial plate.

HARRISON ('26) speak of the entry of the cranial mesectoderm into the formation of the balancer, and ADAMS ('24) and RAVEN ('31 and '33) observe its contribution to the formation of the larval teeth. In these cases the cranial mesectoderm seems to be important in the formation of their mesenchyme, as I have mentioned in the formation of the gills.

V. Summary

The present investigation, which was carried out in *Rana japonica*, concerns the prospective fates of the mesectoderm, with special reference to its cartilage-formation.

1) The mesectoderm begins to migrate in mass downwards in an early neurula-stage and extends considerably before the closure of the neural folds; it does not incorporate in the formation of the neural tube as is the case of urodeles.

2) The migration of the mesectoderm is accomplished simply by the proliferation of its own cells and does not receive contributions from the dorsal portion of the neural tube as in urodeles.

3) Ventrally descending over the mesentoderm, the mesectoderm surrounds each visceral arch and assembles beneath the mesentoderm to give rise to the cartilage. The part remaining over the mesentoderm contributes to the formation of the mesenchyme *in situ*, some of which enters into the gill. Further, some part of the mesectoderm located ventrolaterally to the neural tube is involved in the formation of the cranial ganglia.

4) The mesectoderm transplanted into the brain-cavity directly produces cartilage. The mesectoderm transplanted into the body side (somatic mesentoderm) proliferates in general and produces large pieces of cartilage in the neighbourhood of the myotomes and notochord. The mesectoderm implanted into the endodermal tissues

yields no positive result for the formation of cartilage, in spite of its ganglionic and mesenchymal differentiation. However, in such cases if the transplant is accompanied with the underlying mesentoderm, cartilage always differentiates.

5) The mesectoderm of the frog transplanted into *Hynobius nebulosus* can develop into the cartilage if it is placed in the mesenchyme. The mesentoderm, regardless of the generic difference, is important, though not absolute, as in the case of the brain-cavity, for the cartilaginous differentiation of the mesectoderm.

6) The mesectoderm is determined in the late stage of gastrulation to produce cartilage, and when the neural folds close together it is completely individualized into each group, mandibular, hyoid and branchial. Extirpation of any group results in lack or deficiency of the corresponding cartilage. As the result of extirpation the cartilages such as supra- and infrarostral, MECKEL's cartilage, palatoquadrate and anterior trabecular bar are found to be derived from the mandibular group, hyoid and the first basibranchium from the hyoid group, and all the branchial cartilages are produced from the branchial groups. Among the last named cartilages, the first is only derived from the anterior group, while the rest from the posterior group of the branchial mesectoderm.

7) The basibranchium is of double origin; the large proximal portion which is produced by the union of the hyoid groups on both sides, and the small distal portion which is derived from the mesentoderm in front of the heart. The double nature of the cartilage is proved by the extirpation of all the mesectodermal elements from both sides of the head, with the resulting formation of the mesentodermal second basibranchium alone.

8) The auditory capsule is derived from the cranial mesentoderm and not from the mesectoderm. The fact is well established in both extirpation as well as transplantation experiments.

9) The hypobranchial plate is chiefly produced from the first branchial cartilage but partly also from the second branchial. If the development of the first is checked, the second substitutes for it.

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