EXPERIMENTS ON THE NEURAL CREST
OF THE LAMPREY EMBRYO

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(With Plates 3 and 4 and Three Text-figures)

INTRODUCTION

It is characteristic of the early development of the vertebrate animals that the embryo should be formed largely as a result of successive displacements of the cell population created during and after cleavage. Of these the first bring the endoderm and the mesoderm into what are effectively their definitive positions by paths that vary somewhat from one vertebrate to another. They are followed by morphogenetic movements which see the medullary system, originally external, rendered internal; and this process is substantially constant throughout the phylum. Both by virtue of its massiveness, and by the ubiquity and variety of the tissues to which it gives rise, this system would merit the status of a fourth 'germ-layer' were it not that it is above all from the study of its neural crest component that the limitations of the germ-layer concept in vertebrate embryology has been most strikingly demonstrated (de Beer, 1947).

Nevertheless, as far as our present knowledge goes, the neural crest alone is an embryonic tissue whose fate in different vertebrate classes shows the constancy to be expected of a 'germ-layer'. Thus it certainly gives rise to the spinal ganglia, parts of the dorsal root ganglia in the head, and some of the sheath cells of the peripheral nervous system. In Amphibia it is known to form the bulk of the cartilages of the splanchnocranium and the anterior portion of the trabeculae cranii, and a similar situation probably exists in selachians (Dohrn, 1902), teleosts (Lopashov, 1944) and birds (Goronowitsch, 1892). In Amphibia, birds and teleosts it is an important, if not the sole, source of chromatophores.

For more than one reason it is of interest to know something of the fate of the neural crest in Cyclostomes. In the first place it is possible that the crest was not primitively responsible for the diverse tissues to which it gives rise in the living Gnathostomes. It might therefore be expected that the Cyclostomes would show either the primitive or an intermediate condition, the determination of which would serve as an important pointer to the evolution of the crest. The situation is complicated, however, by the uncertainty that exists on the morphological status to be given to the visceral skeleton and so-called trabeculae cranii in lamprey, the Cyclostome most readily available for embryological study. If, as Rathke (1832) and Balfour (1881) thought, the branchial basket of these animals is not comparable with the visceral skeleton of Gnathostomes, and if, as Sewertzoff (1916) and de Beer (1931, 1937)
have held, the lamprey 'trabeculae cranii' are really anterior parachordals, failure of these structures to owe their origin to the neural crest is not open to a single explanation. These points will be further considered when the results of the present investigation are discussed.

The present state of our knowledge of the fate of the neural crest in lampreys is unsatisfactory. The crest itself was identified by Koltzoff (1901), who thought that it formed part at least of the skeletogenous mesenchyme of the head. Damas (1944), in a recent thorough investigation of the development of the head, comes to the same conclusion, but would derive the 'trabeculae cranii' from ectomesenchyme and not from sclerotome as did Koltzoff. On this last point Johnels (1948) is in general agreement with Koltzoff. Only Hatta (1915) denies a crest contribution to the mesenchyme (which he nevertheless calls mesectoderm!). On the other hand, von Kupffer (1895) and Schalk (1913) claimed to trace the origin of the branchial bars from the ectoderm in the branchial region, and Damas and others agree that there is an extensive placodal contribution to the ectomesenchyme. The material is so unfavourable for normal histological methods that it is doubtful if they will ever permit a clarification of these points.

In respect of other crest derivatives we have only the isolated observations of Bytinski-Salz (1937). After homoplastic transplantation of the anterior neural cord into the blastocoele of a blastula he obtained homoiogenetic inductions of a secondary neural system. Among the induced structures there were, in two individuals, melanophores that he identified as of graft origin. Uncertain as to the possession by lampreys of a neural crest he nevertheless suggested a parallel between this result and amphibian experience.

In this paper are recorded and discussed the beginnings of an experimental approach to the problems presented by the neural crest in the lamprey.

MATERIALS AND METHODS

Eggs of the brook lamprey (*Lampetra planeri* Bloch) were used in these experiments. The early development of this species differs in no known fashion from that of the river lamprey (*L. fluviatilis*). Spawning adults taken from the nest and placed in shallow sinks in the laboratory will, after a short interval, resume nest-building activity and then continue spawning until spent. The great majority of eggs obtained from them are fertile. The culture of these eggs, if they are left in their capsules, presents no difficulties until the stage when, as young ammocoetes, their last reserves of yolk are consumed. The most serious of their requirements seems to be low temperature, for they will thrive only in water kept below 15°C.

The animals used for experiments were decapsulated with fine forceps shortly before suffering operation. This procedure is not difficult at the late neurula stage concerned; about 15% of all animals were damaged and discarded before operation. The naked embryo, until immediately before the age at which it would normally have hatched, is very delicate and can be mortally injured by lengthy contact with glass surfaces. For this reason decapsulation, operation and subsequent culture took place in vessels lined with an agar-agar gel. No specific culture fluid for lamprey
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Embryos has been developed, and so, following the example of Bytinski-Salz, full-strength Holtfreter solution was used for embryos from decapsulation until about 1 hr. after operation. They were then transferred to Holtfreter solution diluted ten times. In this they were kept for 3 days, after which they were transferred to water. All fluids bathing naked embryos contained 1.0 g. of sulphadiazine sodium for every 500 ml.

The operations were performed with the aid of knives ground from steel needles, loops of baby's hair and glass bridges. They involved no technical novelty and need not be described in detail. Post-operative mortality was high mainly because a successful experiment required so long a period between operation and killing for histological study. Mortality is detailed and discussed with the other results.

Animals required for histological examination were killed in Smith's fluid embedded after pre-impregnation with celloidin by the method of Peterfi, and sectioned at 5 or 6 μ. This treatment, followed by staining with Ehrlich's haematoxylin and eosin, gave admirable results for animals of all ages between neurula and young ammocoetes.

RESULTS

(1) Defect experiments

The neural crest in lampreys has not been recognized with certainty at a stage earlier than the late neurula, in which it lies mid-dorsally as an unpaired strip above the medullary cord proper. This, then, represents the earliest stage at which operative procedures on the crest may be practised with confidence. It is, furthermore, impossible to identify the crest cells in dissected embryos (it is difficult enough in sections) after their ventral migration has begun. The short period in which these cells are concentrated in the mid-dorsal line thus determines the stage at which experiments are performed. The advantages offered by amphibian material where the crest may be attacked at any stage between an early neurula and one in which migration is well advanced are here lacking. This limitation is serious in transplantation experiments, but is of less importance for the defect experiments now to be described.

The purpose of these experiments was to determine the derivatives of the neural crest in the head region by examination of young larvae from which parts of that tissue had been removed at an earlier stage. Experiments were performed in two successive years, the results of the first year have been briefly reported in Newth (1950). The two series were treated in a similar way and will be considered together.

The tissue removed in a typical experiment is shown in Text-fig. 1. In so small and pigment-free an embryo the precise extent of the removed mass cannot be judged with certainty. I have, however, satisfied myself by examining embryos killed immediately after operation that the crest was effectively extirpated in the region intended. Indeed, frequently somewhat more of the medullary cord than is indicated in Text-fig. 1 went with it. In order to avoid too great a strain upon any one embryo, no less than to obtain information on the fates of different regions of the head crest, a limited part of the crest was taken in each experiment.
About 120 operations were made, but of these only thirty were successful in the sense that the animals survived sufficiently long to make histological examination worth while. Of these twenty-one were old enough at death to possess differentiated melanophores and recognizable cartilage or pro-cartilage in the visceral arches and at the site of the 'trabeculae cranii'. The other nine had all reached an age at which their cranial ganglia should have been discrete and recognizable.

The post-operative development of the successful experimental animals appeared to be quite normal. The wound was completely healed within 24 hr. in all but one case; in this one the wound area remained recognizable until death as a depression in the dorsal surface of the animal. In some animals the depth of pigmentation on the head seemed to vary from the normal, in four cases it appeared to be less deep, in three others deeper than in control animals. To what extent this is significant will be discussed below. The results of the histological examination of the thirty animals are best analysed with reference to the tissues suspected of crest origin.

The nervous system. Sections of the experimental animals showed an overall normality. Thus in all cases the ectoderm of the dorsal part of the head had healed completely and no trace of scar remained. More importantly the brain was always complete and normal in appearance, clearly as a result of regulative processes in the period immediately following operation. Eyes and otic vesicles were unaffected by the experiment.

It was to be expected that the cranial ganglia of the dorsal series (V₁, V₂, VII, IX and X) might show themselves to be derived in part from the neural crest by deficiencies in the experimental animals. In fact all of these ganglia were affected in one or other animal, the deficiencies, either unilateral or bilateral, being sometimes of a surprising extent. Thus in one animal the vagus ganglion on one side was almost completely absent, though its partner was only partly affected. The section through this animal illustrated in Pl. 3, fig. 4c has been chosen to include the few isolated nerve cells that take the place of the missing ganglion. Normal and experimental
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studies of members of other vertebrate classes have accustomed us to the fact that the respective contributions of crest and placodes to the head ganglia varies considerably, but this result remains extraordinary. The animal is generally normal, and it would appear that one of two explanations of its condition must be accepted. Either the lamprey vagus is derived almost exclusively from the neural crest, or the successful organization of its placodal component is dependent upon the presence of the crest. The second alternative, though out of keeping with amphibian results, seems the more likely. Certainly there is no reason to believe that the vagus in this species lacks fibres generally thought to be of placodal origin (lateralis), and Damas (1944) has described convincingly the process by which placode cells are normally added to the vagal primordium. This result undoubtedly invites further investigation.

In no other case was the reduction in a ganglion so great, though the facialis, glossopharyngeus and vagus were all sometimes very small. Extreme examples of reduction are shown in Pl. 3 with sections through control animals for comparison. The trigeminus was never found to be less than two-thirds of its normal bulk, and if anything the profundus was affected to a still lesser degree. It is difficult to decide with certainty whether a ganglion is affected at all unless the reduction is either very great or else bilaterally asymmetrical. Comparison between control and experimental animals cannot be quantitatively accurate unless the stage of development of each precisely corresponds, and this is not easy to establish. However, I am satisfied that clear defects were to be found in the profundus four times (of which three were bilateral), in the trigeminus twice (both bilateral), in the facialis five times (none bilateral), in the glossopharyngeus five times (two bilateral), and the vagus nine times (six bilateral). The first spinal dorsal root ganglion was affected in three cases. In nine animals no certain defects were observed, and in them effective regulation of the crest must be assumed to have occurred.

The correlation between level of operation on the neurula and subsequent defect was sufficiently good to enable a provisional mapping of the neural crest in terms of its fate. Text-fig. 2 shows the result. It appears from this that the position of the crest components of the cranial ganglia corresponds well with the position of the mesodermal segments with which they will later be associated (for the latter see Damas, 1944). My operations were not, however, sufficiently refined to confirm Damas's observation of a short region above the hyoid segment devoid of neural crest.

Cartilage. Those experimental animals reaching an age at which the first formed cartilages of the branchial basket would normally be present were killed for examination to avoid the risk of their loss from casual death and histolysis. At this stage (6·8 mm. in length, approximately 25 days old) the young animal has chondrified vertical bars with rudimentary epi- and hypotrematic elements in the visceral arches, and well-developed pro-cartilaginous rudiments of the 'trabeculae cranii'.

In no single case was neural crest removal observed to result in deficiencies in these structures.

This result can be explained in several ways. The simplest is to assume that there is no morphogenetic relationship between the crest and the cartilaginous head.
skeleton, but before this assumption is accepted three alternative explanations must be considered. First, it is possible that skeletal structures not yet apparent in animals of this age might have been affected. This is not, in my opinion, a serious objection in the case of the incomplete branchial basket whose morphological integrity is hardly to be doubted, but has some force when applied to the 'trabeculae'. Johnels (1948), who believes that these are primarily somitic in origin, does not exclude the possibility that their anterior parts—in particular the trabecular commissure—are derived from ectomesenchyme. The results of my experiments do not help here, since it is only in older animals that the commissure is formed.

Text-fig. 2. The fate of different regions of the anterior neural crest of the neurula as shown by defect experiments. The approximate extent of the primordia of the ganglia are indicated. P, profundus; T, trigeminus; F, facialis; G, glossopharyngeus; V, vagus; Sp. 1, first spinal dorsal root ganglion.

The second alternative is that effective regulation of the crest has made good the deficiencies in skeletogenous material. This is, of course, quite possible, particularly if considered in conjunction with the third objection, namely, that the high mortality in experimental animals was selective, removing those which had suffered the most radical operations, and hence the most complete removal of crest tissue. Had there been even one animal which showed the slightest deficiency in its head skeleton this possibility would certainly have to be invoked to account for the others which did not. As it is, however, the absence of skeletal defects when considered together with the effects on the neural derivatives of the crest makes it unlikely that this is the proper explanation.

The defect experiments cannot be said to prove the non-crest origin of the lamprey visceral skeleton, but it is fair to conclude from them that it is unlikely that either the branchial basket or the major part of the 'trabeculae cranii' are formed from the neural crest. This conclusion, it must be stressed, does not apply to the larval muco-cartilage.

Melanophores. It has already been mentioned that a few of the experimental animals showed signs of a disturbance of their pigmentation while still alive. This impression was confirmed in study of sections, but it cannot be said that the defect
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Experiments provided satisfactory proof of a relationship between crest and melanophore numbers. In the three animals in which crest removal appeared to lead to an increase in melanophore number the effect was, however, sufficiently striking to be worthy of notice. If later work should show these observations to be trustworthy the explanation is probably to be found in the interesting results of Niu (1947). He found that if the whole of the head crest was removed from Urodele embryos the remaining neural tube over-compensated for the loss by providing many more melanophores than were present in normal animals. Lopashov (1944) also had reason to believe that melanophores arise in the head of teleosts from all parts of the medullary cord.

(2) Transplantation experiments

Living crest cells homoplastically transplanted may be expected, in certain circumstances, to give rise to tissues corresponding to their normal fate. In a series of experiments designed to exploit this situation pieces of head crest were grafted into the flank of other embryos. The grafts consisted of pieces that precisely corresponded to those removed in the defect experiments, and they therefore always contained donor ectoderm, and usually some of the presumptive brain, as well as neural crest (see Text-fig. 3). Host animals were chosen to be a very little younger than donors so that if, after killing, host tissues of suspected crest origin (cartilage, etc.) were seen to be present, time alone should not prevent similar differentiations in cells of graft derivation. Grafts were taken from all regions of the head crest.

In twenty of the twenty-eight experiments made the grafts healed in well, but in the remaining eight they were extruded soon after operation. Of the twenty good hosts eleven survived until an age at which normal cartilage or recognizable pro-

Text-fig. 3. The transplantation experiments. The figure of the donor on the left shows the extent of the tissues grafted in a typical experiment and the region from which similar grafts were taken in the experimental series. The figure of the host on the right shows the graft site and method of grafting.
cartilage of host origin was present in the head region. Some of the other nine have, however, provided information of interest to another problem.

The site of the graft usually remained identifiable in the living animal as a small bulge in the ectoderm. The first indication of morphogenetic activity on the part of the implanted neural crest was the appearance in its neighbourhood of differentiating melanophores. These increased in numbers and came to cover a wider area of the flank as time passed. Their first appearance coincided approximately with that of the host’s melanophores in the head region, that is to say, considerably before the appearance of host melanophores in the trunk. They thus behave ‘herkunftsgemäss’ in respect of rate of development.

Sections showed that the grafts in all cases lay immediately below the ectoderm, between it and the somatopleur. In every animal the graft had given rise to a mass of nervous tissue, sometimes solid and sometimes with a lumen (Pl. 4, figs. 7, 8, 9, 10 and 11). The results of histological examination will be considered under separate headings.

Cartilage. The cartilage of the lamprey embryo is as distinct histologically as that of any other vertebrate, and cells in the pro-cartilaginous stage are also easily recognizable, in particular by their shape and by their large size. In none of the grafts, including those in which host visceral cartilage was well developed, was there any sign of cartilage or of pro-cartilaginous cells. A thorough search through the whole length of the animals failed to reveal cartilage or pro-cartilage except in those places, far removed from the graft site, where normal host skeleton was developing. In view of Raven’s (1933, 1935) results careful attention was given to internal organs (liver, gut wall, etc.) as well as to the body wall.

It is thus very probable that in lamprey cranial neural crest of the late neurula, whether from the pre-mandibular and mandibular (trabecular) region or from the branchial region, will not form cartilage when implanted into the flank of another embryo. This, in my opinion, renders it most likely that the head neural crest in this species does not provide this tissue in normal development, either for the ‘trabeculae cranii’ or for the branchial basket.

These experiments are open to one important criticism in the light of the lessons we have learned from the Amphibia. Contrary to the views earlier expressed by Stone (1926), Raven (1933, 1935), and Ichikawa (1937), it now seems certain that the amphibian crest (or at least that of Urodèles) is not ‘determined’ to cartilage formation in the early neurula. Hörstadius & Sellman (1945) have convincingly demonstrated this point and have suggested a reasonable explanation of the work of other authors that appears to contradict it. It is thus necessary to consider whether the failure of the lamprey crest to develop into cartilage in the conditions of these experiments is due to its determination in normal development at a stage after that at which it was transplanted, e.g. in response to contact with the branchial endoderm. Three points may be made in this connexion. First the operative technique used certainly involved just such damage to underlying host tissues as Hörstadius & Sellman consider provokes cartilage formation in crest grafts to the flank in Amphibia. Secondly, the crest from the donor in the lamprey experiments, although
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not yet in the migratory phase, is, nevertheless, 'older' morphogenetically than that of the open neural plate stages used by these authors. Lastly, and most importantly, Lopashov (1944) has found that in teleosts transplanted neural tissue from the head will give rise to cartilage when transplanted to the yolk-sac or flank of another embryo. His grafts were taken from donors with a solid neural cord and a mid-dorsal neural crest, i.e. comparable to the lamprey donors in point of morphogenetic age.

These considerations do not completely eliminate the doubts to which the results of Hörstadius & Sellman must give rise, but they do, in my opinion, enable us to say that it is extremely unlikely that the cranial neural crest in lamprey is skeletogenous. Taken in conjunction with the results of the defect experiments the matter seems to me to be hardly in doubt. If grafts of head crest to the head region of other embryos also give negative results the demonstration will be complete. This test I hope to apply in the coming breeding season.

Mesenchyme and chromatophores. Sections revealed isolated cells in the neighbourhood of each graft lying between the lateral plate mesoderm and the ectoderm of the host. In the younger animals it was not possible to identify them from their appearance, they might equally have been neuroblasts or mesenchyme cells. In the older ones they could be seen to be mesenchymatous. In none of the experiments were they very numerous, but some could always be found. That they originated in the graft is most likely as sections through comparable regions of control animals failed to show similar cells. This serves to confirm Damas's observations, based on serial sections of normal material, that the head crest has an ectomesenchymal component. It does not, however, mean that the crest is the sole source of ectomesenchyme in the head. On the contrary, Damas has shown that both placodes and stomodaeal invagination contribute largely to this tissue, and it may well be that the diverse cell types that differentiate from the ectomesenchyme are strictly related to its several sites of origin.

The differentiation of melanophores in the region surrounding the graft is of interest from several points of view. In the first place it must be said that in the light of amphibian and avian experience it cannot be doubted that they are of graft origin. They thus provide a good marker of the migratory capacity of crest cells. Damas has, indeed, described this migration from his sections, but the difficulties in interpreting sections of young lamprey material makes direct confirmation of value. The extent of the migration from the graft site can be seen in Pl. 4, fig. 6, which shows that the distance over which crest cells can migrate is quite as large as that of their amphibian analogues.

As with the mesenchyme, however, it must not be assumed that the crest is necessarily the sole source of melanophores in normal development. Lopashov (1944) has found indications that in teleosts the whole brain may provide them, and this may be true of lower vertebrates generally, while Niu (1947) has shown that the brain will do so in Amphibia under experimental conditions. The positive result of these transplantation experiments as far as the melanophores are concerned has as its most important consequence the demonstration that a migratory neural crest with components destined to a non-neural fate is a property of craniate vertebrates, both
jawed and jawless. It is thus almost certainly an archaic feature characteristic of the common ancestors of the Agnatha and Gnathostomata.

The lumen of the neural canal. Holtfreter (1933, 1934) has shown that in Amphibia the disposition of the cell bodies in the neural tube, and the shape and position of its lumen, are determined by the nature of its environment. Thus an isolated embryonic neural ‘tube’ is, in fact, solid with the cell bodies at its periphery; one surrounded by mesenchyme has a central lumen, circular in cross-section, with the cell bodies centripetally arranged; one in contact with a block of somitic muscle has its lumen displaced to the opposite side; while one in contact with a chorda has its lumen elongated in cross-section with the long axis pointing towards the contiguous tissue. It is easy to see that these results explain the form of the normal spinal cord in terms of its relations with the flanking myotomes and the underlying notochord.

Huxley & de Beer (1934) have suggested that these results may shed light on the evolution of the vertebrate brain, in the sense that if they were generally valid an environment of myotomes (as is suffered by the anterior neural tube of Amphioxus) would militate against the formation of a vesicular, thin-walled brain. It is therefore of interest to observe that in the lamprey those grafts which differentiated into neural masses with a lumen had the form that Holtfreter’s results would have led one to expect (Pl. 4, figs. 7, 10 and 11). In them the wall of the neural vesicle which lies against the lateral plate mesoderm is always thick, while that lying against mesenchyme or ectoderm is thin. In one case (Pl. 4, fig. 11), in which the vesicle had two opposed walls in contact with the ectoderm, a structure closely resembling the normal neural tube resulted. It thus appears that endodermal tissues may exercise an effect similar to that of musculature in this process. The grafts which remained solid are not relevant to this problem as they were undoubtedly ganglionic in nature, having few or no cells from the brain or spinal cord proper (Pl. 4, figs. 8, 9). We are again forced to admit that a developmental process common to both Amphibia and lamprey was probably shared by their ancient common ancestral stock. To this extent the value of the suggestion of Huxley & de Beer is enhanced.

DISCUSSION

That the possession of a migratory neural crest with components destined to non-neural fates is a feature not only of Gnathostomes but of all the craniate chordates is not surprising. The establishment of the capacity of the lamprey neural crest to form melanophores assumes additional importance, however, when we come to consider its apparent incapacity to form cartilage. This failure cannot be associated with lack of opportunity, in the sense that crest cells migrate to regions in which visceral cartilages are formed, the histogenesis of cartilage and the formation of melanophores in the branchial arches taking place contiguously and almost simultaneously. The actual origin of the visceral skeleton in lamprey cannot yet be stated with certainty, but the admirable observations of Damas (1944) command attention. He finds that the branchial bars (and also the ‘trabeculae cranii’) are indeed formed from ectomesenchyme, but that this tissue has a composite origin being derived both from the crest and from an extensive placodal contribution. It
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thus seems that the earlier conceptions of von Kupffer (1895) and Schalk (1913) may well prove to be correct. They found that the branchial skeleton was formed from cells given off from the deeper layers of the overlying ectoderm (branchiodermis of Schalk). It is almost certain that Hatta’s (1915) belief in the mesodermal origin of these cartilages is ill-founded.

As to the ‘trabeculae cranii’ Damas’s observations have not been confirmed by Johnels (1948), who believes, as did Koltzoff (1901), that they are formed from the sclerotome of the anterior head somites. The present work is, of course, quite consistent with this belief.

Now if it be true that the ‘trabeculae cranii’ of lamprey are derived from sclerotome, and that the branchial basket is not of neural crest origin, the homologies of these structures with their gnathostome analogues is called into question. It must, of course, be recognized that embryological origin cannot be the sole criterion of homology, and that it is possible to accept the homology of elements of the lamprey and gnathostome splanchnocranium without reference to embryology. It so happens, however, that these homologies have for some time been suspect on morphological grounds. We may therefore fairly cite the embryological evidence against them without thereby claiming that it is alone sufficient to determine the issue.

The evolution of the gnathostome visceral skeleton is now well established in a general sense from the Aphetohyoidean grade of organization onwards. The discovery that the anterior portion of the trabeculae cranii in Amphibia share the neural crest origin of the cartilages of the jaws and visceral bars adds strong support to the view of Allis (1923) and de Beer (1931) that the trabeculae represent skeletal elements from a pre-mandibular visceral arch.* It is thus to be considered probable that there existed pre-Aphetohyoidean gnathostome ancestors which possessed one functional gill slit before the spiracle. The backward growth of the mouth must, of necessity, have obliterated this slit, while the skeletal element lying in the pre-mandibular arch (or part of it) then came to serve the needs of the neurocranium as a trabecula. Holmgren (1949) has put forward objections to this view on the basis of his studies of the origin of the trabeculae in fishes, but the balance of considerations favours it.

It follows from this that if the lampreys possess true trabeculae cranii they, or their ancestors, must possess or have possessed a series of gill bars of the same nature as those of the Gnathostomes, i.e. homologous with them. Certainly the lamprey condition in which the first functional gill opening is post-spiracular is secondary, for, as Stensiö (1927) has shown, the Osteostraci are not only the most likely lamprey ancestors, but also possessed two more anterior openings. Thus lamprey ancestors may be assumed to have had a functional pre-mandibular arch between the mouth and the first gill-opening. But is the agnathan visceral skeleton to be homologized with that of the Gnathostomes?

* Serial homology is clearly a reality though no more to be considered as a rigid system than normal homology. In particular the weight to be given to embryological origin in determining serial homology must be separately estimated in every case. The experiments of Bijtel (1931) and Ford (1950) show that such serially homologous structures as myotomes may have diverse anlagen in a single individual.
Rathke (1832) first opposed the ‘external’ branchial basket of lampreys to the ‘internal’ gill bars of Gnathostomes. In this he has been followed by some later workers, notably Balfour (1881). Others (Dohrn, 1902; Balabai, 1937) have sought to homologize them. The literature on this subject is too extensive for review here, but it must be mentioned that in two cases it has been suggested that elements of the non-prevailing type of visceral skeleton can be found in living forms. Thus it has long been thought that the extra-branchialia that occur in some selachians were parts of an external arch system, and Holmgren (1946) has recently discovered structures in a *Myxine* embryo which he regards as elements of the internal arch skeleton of the mandibular, hyoid and first branchial segments. These results of Holmgren are of the greatest interest, but, unfortunately, only serve to emphasize the need for a thorough knowledge of progressive stages in the development of *Myxine*.

While the morphological status of both the extra-branchialia and Holmgren’s internal arches in *Myxine* cannot yet be regarded as established, the more general statement of the non-equivalence of the major elements in the cyclostome and gnathostome visceral skeleton commands respect. Of the two the cyclostome splanchnocranium is the more easily interpreted. Following Stensiö (1927) and Holmgren & Stensiö (1936) we may regard the lamprey branchial basket (and probably the muco-cartilaginous elements in the Ammocoetes) as a remnant of the originally continuous endoskeleton of the Osteostraci. Damas (1944) has gone further in suggesting that the distribution of the ectomesenchyme in lamprey embryos before parts of it have given rise to cartilage or muco-cartilage is such as to permit comparison between this embryonic tissue and the endoskeleton of the adult Ostracoderm. Be this as it may, there is no reason to suppose that lampreys ever had a system of discrete bars as a gill skeleton. The similarities in gill function between *Cephalaspis* and lamprey make it quite reasonable to assume that the visceral skeleton of the latter is derived from that of the former by the simple loss of most of the primitively continuous structure. The fact that the first elements of the branchial basket to chondrify are the vertical rods does not at all justify their comparison with the bars of Gnathostomes made by Sewertzoff (1916).

Now this interpretation does not preclude there being present in the lamprey some equivalent of the skeleton of its ancestor’s pre-oral arches. Sewertzoff (1916) even claimed to find elements of three pre-mandibular arches, but this is so at variance with all our present knowledge of the anterior region of the craniate head that it need not be considered further. Of the workers who have studied this problem in recent years none has suggested that the ‘trabeculae cranii’ are pre-mandibular arch elements. Indeed, the evolution of the Agnathan head has not been accompanied by that backward growth of the mouth typical of Gnathostomes, and consequently there is less reason for supposing that visceral elements in the pre-mandibular region should be ‘pushed’ upwards to become attached to the neurocranium as trabeculae. There is, in fact, no reason at all for believing the ‘trabeculae’ of lampreys to be visceral.

When we come to the Gnathostomes difficulties arise. It is almost certain that the osteostracan pre-spiracular gill opening is a primitive craniate feature, the loss of anterior gill openings in Agnatha and Gnathostomes being an example of major
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parallel evolution. This being so, are Osteostraci possible Gnathostome ancestors? If
the interpretation of the trabeculae as visceral elements of an internal pre-mandibular
arch skeleton is correct this seems unlikely. Rather should we expect the Gnathostome
ancestor of the grade possessing a pre-spiracular gill opening to have a gill system of
the slit type with a visceral skeleton composed of bars not wholly unlike the branchial
bars of living fish. In the absence of fossil evidence it would be wrong to assert
that this was so. All that can be said is that the present state of our embryological
knowledge supports this conception, but that the much-to-be-desired knowledge of
the development of Myxine may yet clarify these points in some different sense. In
particular, it must be borne in mind that the visceral endoskeleton of the ostracoderms
may have two distinct descendants: the dorsal endocranium becoming the lamprey
branchial basket, and the ventral the gnathostome system of bars.

SUMMARY
1. The fate of the neural crest of the head in Lampetra planeri has been studied by
experiments involving the removal or the homoplastic transplantation of short
regions of this tissue.
2. The results show that the neural crest of lamprey is normally destined to form
part at least of the dorsal root ganglia, and that it probably gives rise to most, if not
all, of the melanophores and to some of the ectomesenchyme.
3. No evidence that the neural crest of the head was the source of the 'trabeculae
cranii' or the cartilages of the branchial basket was found. It is concluded that these
structures are most probably not crest derivatives.
4. The phylogenetic significance of the embryology of the lamprey 'trabeculae
cranii' and visceral skeleton is discussed.
5. The transplantation experiments provided some evidence that the form of the
neural tube in lamprey is determined in the same manner as that established by
Holtfreter (1933, 1934) for Amphibia.

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EXPLANATION OF PLATES

PLATE 3

The four defect experiments here illustrated are chosen to show the most extreme reduction in the cranial ganglia V, VII, IX and X. On the right are shown sections through a control animal 7·0 mm. in length, in the middle are shown the operations, and on the left the resulting deficiencies.

Fig. 1. a, defective profundus ganglion in experimental animal. The section is not quite transverse, only the left side of the animal (right in picture) should be compared with control. b, operation. c, the profundus, lying above the eye, in a normal animal.

Fig. 2. a, defective posterior lobe of facialis ganglion on left side (right in picture). b, operation. c, the normal appearance of the facialis ganglion.

Fig. 3. a, defect in glossopharyngeus ganglion in experimental animal. The section is not quite transverse, only the left side of the animal (right in picture) should be compared with control. b, operation. c, the normal appearance of the glossopharyngeus ganglion.

Fig. 4. a, the vagus region of an experimental animal. Note reduction in animal’s left ganglion and the almost complete absence of the right-hand one. b, operation. c, control animal in the region of the vagus ganglion.

PLATE 4

Fig. 5. The head neural crest as seen in a transverse section through a neurula (cf. Text-fig. 1).
Fig. 6. Homoplastic graft of head neural crest into flank at the neurula stage has resulted in development of melanophores in the graft region of the host. The graft can be seen as a small protrusion lying ventrally.

Fig. 7. Neural vesicle of graft origin seen in section. It is flanked by host lateral plate mesoderm on one side and by mesenchyme and ectoderm on the other.

Fig. 8. Neural mass of graft origin seen in section. It has no lumen. One or two ectomesenchymal cells of graft origin can be seen on either side of the neural tissue.

Fig. 9. Neural mass of graft origin seen in section. It has no lumen. The dark cells at its lower edge are melanophores.

Fig. 10. Neural vesicle of graft origin. Ectomesenchyme cells are present between the host lateral plate and the ectoderm.

Fig. 11. Neural vesicle of graft origin. It is flanked by host endoderm and is in contact with the ectoderm above and below.