THE MECHANICS OF VERTEBRATE DEVELOPMENT

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(With 11 Text-figures.)

CONTENTS.

1. INTRODUCTION.

It is proposed in this article to review the work which has been done and the results which have been achieved in the experimental study of the early development of vertebrate animals by the already large number of investigators in all quarters of the globe.

Roux, to whom the science of developmental mechanics owes so much, distinguished two main periods in development: an earlier one in which the organs appear without function, and a later one in which function conditions the further development of the organs. These pages will concern only the earlier period. The experimental results obtained in connection with functional differentiation and adaptation are therefore purposely excluded from this review, as also are those dealing with hormone action, the action of abnormal environments and with regeneration.

The present scope therefore is limited to an analysis of the processes whereby in normal development the various rudiments of the vertebrate embryo are

determined and make their first appearance. The strides which have been made in this study are due to the invention or perfection of new methods. Experiments in this field are no longer "mass-performances" with statistical results, but are more often microsurgical operations on individual embryos. The fine needle as used by Roux (1895 *b*) and others, enables a definite region to be destroyed. A child's hair can be used as a ligature to separate two blastomeres, as Herlitzka (1897) and Spemann (1921 *a*) have shown. To the latter is also due the micropipette with which definite and minute regions of an embryo can be removed and transplanted to another embryo with great precision. Glass needles, hair loops, very fine knives, Peterfi's (1924) microcauteriser and Chambers' (1922) microdissection apparatus complete this microsurgical equipment.

Other methods have for their object not the removal but the identification of certain regions so as to be able to follow their displacements during development. For this purpose, the methods of *intra vitam* staining elaborated by Goodale (1911), Detwiler (1917) and Vogt (1925) are pre-eminent.

Yet other methods aim at studying the behaviour of definite regions when completely isolated from the organism. To this end they may be explanted as tissue cultures *in vitro* in suitable media, or in the case of birds, grafted on to the chorio-allantoic membrane of another developing egg, by methods due to Peebles (1898), Murphy and Rous (1912), Murphy (1916), Danchakoff (1916) and Minoura (1921). *In vitro* methods for such morphogenetic rather than histological study have been elaborated by Braus (1911), Harrison (1912), and by Strangeways and Fell (1926).

The material worked upon necessarily bears a relation to the methods mentioned, and consists chiefly of frogs, toads, newts, chicks and a few fish. Nevertheless, so many "complex components" of development have been discovered that it is possible to obtain a general idea of the causal connections between many of the important developmental processes.

For previous general or partial surveys of this field the reader may be referred to the following works: Jenkinson (1913, 1917), Dürken (1919), Petersen (1923, 1924), Brachet (1917), Spemann (1919, 1924), Mangold (1925), Huxley (1924), Duesberg (1926), de Beer (1926), Przibram (1926).

It goes without saying that, in what follows, the comparative embryology of vertebrates is taken for granted, except in so far as certain experiments have shed more light on various processes.

2. PROLEGOMENA.

It is necessary in the first place to have a clear idea of the structure and potencies of the egg, that of Amphibia being chosen as an example. When laid, the frog's egg although spherical possesses an axis, since yolk is accumulated at one (the vegetative) pole, and protoplasm and pigment at the other (the animal) pole. Yolk having a higher specific gravity than protoplasm, the axis of the egg is normally vertical with the animal pole on top. Gravity is however not responsible for the formation of this axis, as the following experiment of Roux (1895 *a*) shows.

The action of gravity can be eliminated by putting the egg in a clinostat which

revolves slowly about a horizontal axis. The eggs are constantly tumbling over one another and therefore do not present the same pole to the centre of the earth for any length of time. Nevertheless normal larvae develop. The axis must have been determined previously, before the egg was laid. Gravity cannot be invoked at these early stages either, for in the ovary there is no definite orientation. Bellamy (1919, 1921) has been able to show that the capillaries of the follicle probably determine the axis of the egg since at early stages the animal hemisphere is that to which arterial blood is supplied, while the venous blood is removed from the region of the vegetative hemisphere. This is not necessarily true for later stages however. The prime differentiation is therefore impressed from without, for the animal pole will become the head of the embryo.

The bilateral symmetry of the egg is brought about for the most part by the entrance of the sperm (Roux, 1903; Jenkinson, 1909) in normally fertilised eggs. In frogs, the grey crescent is formed at the meridian antipodal to that where the sperm enters, by retreat of pigment to the interior. That the two events are connected follows from Herlant's (1911) observation that in frogs' eggs into which two sperms have entered, the grey crescent appears at the antipode to the meridian half-way between the points of entry of the two sperms. At the same time Jenkinson (1909) showed that the plane of symmetry of the egg might in certain cases be determined by incident light and by gravity. Both these factors are external to the egg. Since the dorsal lip of the blastopore begins to form in the centre of the grey crescent, it may be said that the sperm fixes the "Greenwich" meridian of the egg (again from without) and determines the future dorsal, ventral and right and left sides. No grey crescent is found in the newt's egg, but a region with characteristic pigmentation appears in the axolotl after fertilisation (less distinctly in *Triton*), in which the dorsal lip of the blastopore later arises. This was proved by *intra vitam* staining marks by Vogt and Goerttler (quoted by Bautzmann, 1926).

The plane of the first furrow is at right angles to the first spindle and this spindle is at right angles to the later or "copulation" path of the sperm (Roux). If the "penetration" and "copulation" paths of the sperm are in the same straight line, then the plane of the first furrow will coincide with the plane of bilateral symmetry. If on the other hand the copulation path is deflected, the plane of the first furrow may make any angle with the symmetry plane. Jenkinson (1909) was able to study these relations in horizontal sections of the two-cell stage, the path of the sperm being indicated by a trail of pigment. Roux's method was to cause the sperm to enter the egg in a definite meridian by means of experimental devices such as a fine pipette or a thread leading the sperm to the egg.

In newts, by placing a ligature round the egg in the plane of the first furrow and observing the relation which it makes with the dorsal lip of the blastopore when it appears, Spemann found that the first furrow tends either to coincide with or to be at right angles to the plane of bilateral symmetry. By means of *intra vitam* stains used as reference marks, Vogt (1923) concludes that there is more irregularity in the relation of the planes than Spemann supposes. The importance of these matters will be apparent in what follows.

With regard to the normal process of gastrulation, experiments have settled two important points; first the relative contributions of ingrowth and epiboly to gastrulation in Amphibia, and the question of concrescence as a method of closure of the blastopore.

In *Amphioxus*, the egg of which contains little yolk, gastrulation takes place by invagination as a result of which the diameter of the blastopore is at first nearly that of the whole blastocoel, and it subsequently becomes reduced and closed by growth of the blastopore rim. In Amphibia, epiboly takes place certainly as a means whereby the dark cells of the animal hemisphere come to cover the lighter coloured

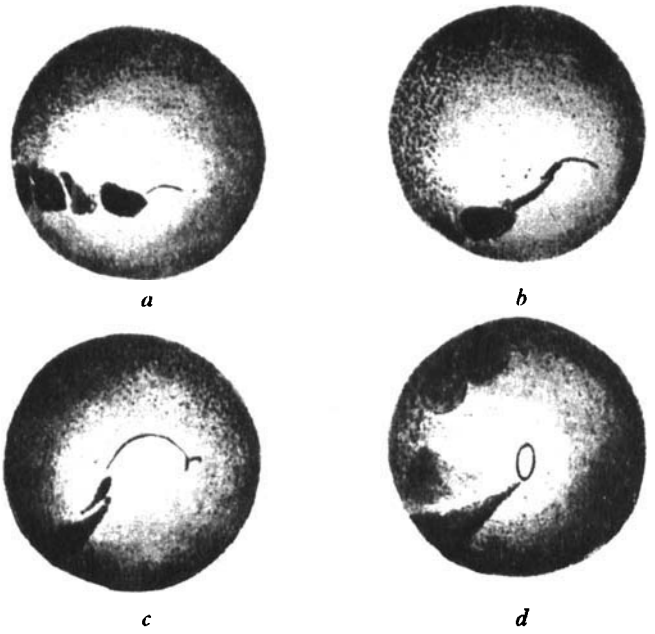

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Fig 1. Embryo of *Pleurodeles* during gastrulation with *intra vitam* reference marks. The marks get carried to the rim of the blastopore, turn in over its edge, and are seen in *d* by transparency through the ectoderm, moving away from the blastopore on the inside. (From Goerttler.)

cells of the vegetative pole, but owing to the large amount of yolk present, typical invagination as in *Amphioxus* cannot occur, and some have doubted whether it occurs at all. By placing reference marks of *intra vitam* stains on the surface of blastulae of *Triton* and *Pleurodeles*, Goerttler (1925) observed that they disappeared over the edge of the lip of the blastopore and could be followed by transparency moving away from the blastopore on the inside. There is no doubt therefore that ingrowth does occur, a matter of great importance in subsequent differentiation. Vogt (1922 *c*) observed that the material at the rim is constantly changing by rolling in, and is supplied by new cells which move towards the blastopore on the outside. The maximum ingrowth takes place in the mid-dorsal line, and *intra vitam* reference marks show that on the surface, in the region between the animal pole and the dorsal lip of the blastopore, pronounced stretching takes place in the meridional plane.

To a lesser extent the stretching and rolling in takes place all round the blastopore rim. The nearer a mark is to a rim of the blastopore at the start, the further forward does it get pushed inside.

By means of transplantation experiments to be detailed later, Mangold (1924) showed that the different regions of the blastula have certain tendencies towards "mass movements," the results of which are the process of gastrulation. So the cells of the animal hemisphere, especially on the dorsal side, tend to grow and increase their surface; those in the region of the future blastopore rim tend to grow in, while the vegetative pole regions are more passive. It is worth while noticing that as the lips of the blastopore, the ring of overgrowth, are below the equator, the process of epiboly entails a contraction of this ring corresponding to the decreasing diameters of latitudes approaching the vegetative pole. Vogt (1922 *a*, *b*) removed a portion of the roof (animal pole) of the blastula of *Triton*. The gap closed by approximation of the sides of the wound. But by this process the future ring of overgrowth was drawn up above the equator. When gastrulation started this rim attempted to grow over and at the same time to decrease its diameter. The result

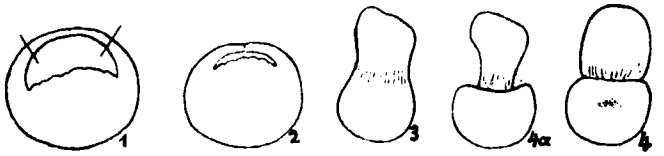

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Fig. 2. Diagrams illustrating experiments on *Triton* embryos. 1. A portion of the roof of the blastula is removed; 2. The wound closes over by approximation of the sides. 3. A constriction develops, due to the decreasing diameter of the presumptive blastopore-rim, which is drawn up above the equator by the closure of the wound. 4*a*. The constriction becomes accentuated 4. A separate invagination takes place in the region of the yolk cells. (From Vogt.)

was a constriction causing the embryo to resemble an hour-glass. At the same time, beneath this rim which represents the margin of overgrowth, and separate from it, a simple pit is formed by invagination in the yolk cells. By the experiment, therefore, these two processes were more or less dissociated. On the other hand, by treating frogs' eggs with LiCl, $CaCl_2$, sugar or sodium acetate, Giersberg (1924) obtained gastrulae in which invagination had occurred, but no epiboly, for the blastopore remained enormous.

After completion of gastrulation, the continuance of the movements which brought it about play an important part in the formation of the tail bud. (Vogt 1926.) As regards the supposed closure of the blastopore by the concrescence in the middle line of laterally situated material, Goodale (1911), by means of *intra vitam* staining, found that the movement of material in *Spelerpes* and *Amblystoma* took place along meridional lines, *i.e.* parallel to the mid-dorsal line, and not towards it. The same thing has been shown by Vogt (1922 *c*) and Goerttler (1925) for *Triton* and *Pleurodeles*, and by Smith[1] (1914) for *Cryptobranchus*. In selachian embryos, Kastchenko (1888) found that injury to the edge of the blastoderm was

[1] Smith's interpretation of his results is slightly different.

not carried to the middle line, but remained on one side. Lewis (1912) working on *Fundulus* found the same thing; indeed here a side of the germ ring can be removed without interfering with the blastopore or the embryo. If concrescence occurred, injury to the dorsal lip of the blastopore in the middle line should not prevent the more lateral tissue from growing towards the middle line further back; Kopsch (1896) found in the trout that it did prevent it. Lastly, a very elegant experimental disproof of concrescence has been given by Vogt (1923). If concrescence does not occur, then those cases of spina bifida, when the blastopore, *e.g.* of Amphibia, does not close properly must be due, not to the failure of the lateral

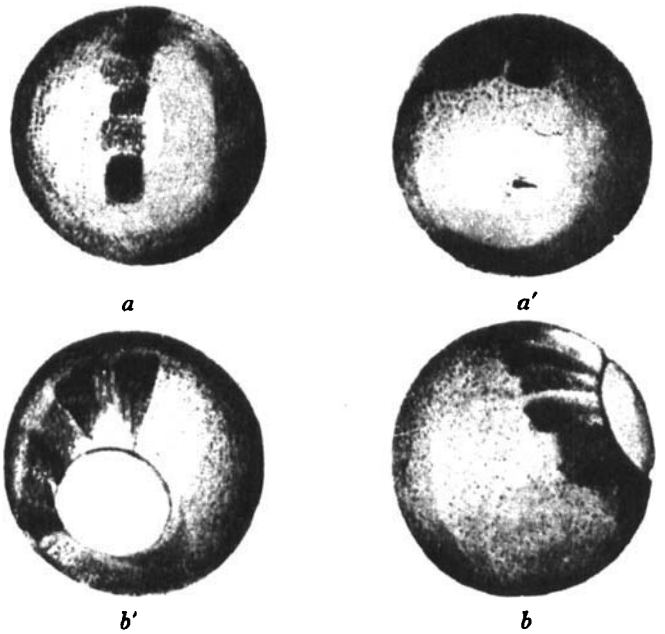

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Fig. 3. Embryo of *Pleurodeles* with *intra vitam* reference marks. *a* and *b*, lateral views; *a'* and *b'*, views from the vegetative pole; *a*, early stage; *b*, late stage of gastrulation. The marks move along meridional lines to the blastopore rim. (From Goerttler.)

tissue to reach the middle line, but to the displacement to the sides of tissue normally in the middle line. To prove this, an *intra vitam* reference mark was placed in the centre of the dorsal lip of the blastopore at the outset of gastrulation in a newt's egg. Spina bifida or "Asyntaxia" was then induced by artificial means (certain solutions are capable of effecting this) and it was found that the reference mark had been split into two and displaced on each side away from the middle line.

As the diameter of the blastopore decreases during gastrulation, so points on its rim get nearer together. This is not concrescence but convergence.

The *intra vitam* staining method can be used in the chick, as by Wetzel (1925), who showed that a mark at the posterior edge of the blastoderm stretches forward in the formation of the primitive streak.

3. DETERMINATION BEFORE AND DURING CLEAVAGE.

It is necessary to consider the facts relating to the potency and restrictions of potency, or determination, of the egg and the blastomeres into which it divides during cleavage.

Roux (1895 *b*) killed one blastomere of the 2-cell stage of the frog, by means of a hot needle. The result of this classical experiment was that the remaining blastomere developed into a half-embryo; usually a hemiembryo lateralis, or in some cases a hemiembryo anterior. It looked as if the blastomere gave rise only to those structures which it would have produced in normal development, and that it was already determined as regards its fate. On this experiment and on Weismann's speculations, rests the Roux-Weismann theory of mosaic development, which regarded the division of nuclei as responsible for the differential determination of the different blastomeres. The fact that some of Roux' hemiembryos later on completed themselves by postgeneration does not affect the present argument.

Hertwig (1893) challenged Roux' conclusions and showed that differentiation could not rest on unequal nuclear division between the blastomeres. By compressing frogs' eggs in different ways, he completely altered the normal distribution of the nuclei and nevertheless obtained perfect embryos. From his experiments of injuring one blastomere he concluded that Roux' half-embryos were produced owing to the dead blastomere remaining in contact with the living one, and prophesied (successfully) that if the dead one were removed, the other would reveal itself totipotent, and produce a whole embryo. The variability of the results of killing one blastomere also weighed against the probability of a determined mosaic development.

The analysis was carried a step further by Schultze (1894), who reversed frogs' eggs at the 2-cell stage so that their light-coloured vegetative poles were uppermost. The result was double monsters, *i.e.* each blastomere produced much more than half an embryo, which was its normal prospective fate. Wetzel (1895) confirmed these results and showed that after reversal a reorganisation of each blastomere takes place, the yolk sinking down along the cell-wall dividing them and the protoplasm rising, as Born (1885) had shown to be the case with the egg. These double monsters have a common blastopore. The reorganisation and rearrangement of the contents of the blastomeres are therefore responsible for restoring to them the original potency of the egg.

Morgan (1895) adapted these results to Roux' experiment and showed that when one blastomere was killed, if the other were left animal pole uppermost it developed into a half-embryo, if it were reversed so as to lie vegetative pole up it developed into a whole embryo of small size.

Hertwig's prophecy was verified by McClendon (1910), who removed one blastomere of the 2-cell stage of the frog *Chorophilus triseriatus* by sucking it out with a fine pipette. The remaining blastomere became a small whole embryo.

There is no doubt therefore that in the frog the prospective potency of the blastomeres is greater than their prospective fate, and the potency of the blastomeres of the 2-cell stage is, except for one important reservation, equal to that of the

egg. In order to develop, a blastomere must contain a portion of the grey crescent, or future blastopore lip. Brachet (1917) showed that Schultze's double monsters are only obtained if the planes of the first furrow and of bilateral symmetry nearly coincide; the result of which is that each blastomere has some of the grey crescent. Again, it has already been mentioned that such monsters have a common blastopore.

There is therefore an important determination already present in the frog's egg; the grey crescent. In addition there is evidence of a reversible determination of certain regions. Brachet (1905) observed that when one blastomere was killed and the other allowed to develop without disturbing it, the resulting half-embryo was orientated with regard to the grey crescent. It was either a lateral half, an oblique half or an anterior half, according to the angle between the planes of the first furrow and of symmetry. Brachet (1906) further discovered that by making a small injury in an egg up to 45 minutes after fertilisation no effect was produced; between 45 and 60 minutes after fertilisation, it resulted in an asymmetry on the injured side, which means that a quantitative deficiency on that side had not been made good. Injury from 60 to 90 minutes after fertilisation resulted in qualitative deficiency of some region. Morgan (1905) removed material from the animal pole at the 2-cell stage and observed that the resulting embryos were deficient in the dorsal region. By acting with ultra-violet light on frogs' eggs in the 1- or 2-cell stage, Baldwin (1915, 1919) produced quantitative defects (asymmetry) without qualitative or histological deficiencies.

Dürken's (1925 *b* and 1926) experiments are of the greatest interest in this connection. In order to answer the question whether tissues which have not been subjected to the action of the blastopore lip can differentiate, he took pieces from the region of the animal pole of blastulae and gastrulae of *Rana fusca*, and grafted them into the cavity of the orbit of older embryos. Pieces from blastulae differentiated (of course abnormally yet unmistakably) into notochord, cartilage and ganglion cells which formed ganglion-like masses. In another case he obtained differentiation of a portion of the brain and auditory vesicles. A piece from a young gastrula gave a limb-like structure containing cartilage, bone, muscle, nerve cells, blood vessels, glands and connective tissues. The tissues of the host took no part in these structures.

These experiments show that in addition to the irreversible grey crescent, frogs' eggs have from an early stage certain determinations which are reversible at first (*teste* whole embryos from one blastomere) and which are *cytoplasmic*, not nuclear.

In the newt's egg, Herlitzka (1897) showed that the blastomeres of the 2-cell stage could be separated by ligaturing between them with a fine hair, and that each could produce a perfect embryo. These results have been confirmed and amplified by Spemann (1901 *a*, 1902, 1903), who found that incomplete constriction produced duplicitas anterior, and that complete constriction could be followed by the formation of a pair of normal small embryos, even as late as the early gastrula stage, provided always that the plane of constriction (*a*) was meridional, and (*b*) coincided with that of symmetry. In other words each blastomere or half-gastrula must contain a portion of the future dorsal lip of the blastopore. If the constriction is

made transversely, one product will contain this blastopore region and will develop: the other will lack it and will never develop beyond forming the three germ layers. After obtaining separate blastomeres of the 4-cell stage, Ruud (1925) observed that two of these developed into normal miniature larvae whereas the other two (which lacked the blastopore-lip-region) did not develop at all.

The converse experiment to obtaining wholes from portions is to obtain a single whole from two conjoined embryos. This remarkable feat was performed by Mangold (1920) by placing two 2-cell stage embryos of *Triton* crosswise over one another, after freeing them from their membranes. If the two blastopore-dorsal-lip-regions did not come to lie adjacent to one another, the result was a double monster. If on the other hand they did come together, a single normal giant embryo was formed.

These results show that in the newt's egg the region of the future dorsal lip of the blastopore is the only determined region, before and during cleavage. No other regions are determined at these stages. Except for the dorsal-lip-region, newt's eggs and blastomeres regulate. Still more so do the blastomeres of the fish *Fundulus heteroclitus*, the potencies of which were tested by Lewis (1912). Injury with a needle to the undivided egg within 2 hours of fertilisation gave rise to normal small embryos. Destruction of one blastomere of the 2-cell stage does not prevent the other from forming a normal small embryo. At the 4-cell stage removal of any one blastomere results in the remaining three forming a normal embryo; and if two blastomeres are removed, the remaining two, provided that they are not a diagonal couple, will do likewise. Even at the blastoderm stage injuries fail to induce qualitative abnormality in the resulting embryos.

The result of these enquiries is to show that in the amphibian egg, the region destined to form the dorsal lip of the blastopore is a determined region of extreme importance, and that at the early stage here considered other determinations are either non-existent (newts and *Fundulus*) or labile (frogs) up to a point. At all events, such determinations are cytoplasmic and not nuclear. This fact, which has now been established in most groups of the animal kingdom, is of the greatest importance. No demonstration of it could be more elegant than that of Spemann (1914). By placing a ligature round an egg of *Triton* before cleavage begins, and dividing the egg incompletely into two halves, the nucleus can be confined to one side of the constriction. After the nucleus has divided a certain number of times on that side the constriction can be released to allow one product of division to cross to the other side. If the constriction was in the symmetry plane, each of the lateral halves can develop, whether it contains one sixteenth or fifteen sixteenths of the original cleavage nucleus (Spemann, 1924). If, however, one sixteenth of the original cleavage nucleus is allowed to cross the bridge from the ventral to the dorsal half, the latter will not develop. This means that after the nucleus has divided three times in the ventral half, its products are incapable of functioning normally in the dorsal half. That this incapacity is due to a cytoplasmic effect on the nucleus is proved by the fact that, in lateral halves, a one-sixteenth nucleus is perfectly capable of functioning so as to produce a normal embryo. This result is obtained

by severing all connection between the blastomeres after the nucleus has passed across. If they are allowed to remain in contact a double monster is produced, the components of which differ in "age."

Lastly, if a (lateral or dorsal) half, from which the cleavage nucleus and its products have been completely excluded, happens to contain an accessory sperm, it will develop into a haploid but otherwise normal embryo.

Haploid embryos can also be obtained by exposing the eggs or the sperms to radio-active substances before fertilisation. G. Hertwig (1913) obtained embryos from eggs of *Rana esculenta* and *Bufo vulgaris* which had been fertilised with irradiated sperm of *Rana fusca*. P. Hertwig (1913) showed that after having been radiated the sperm nucleus plays no part in such development, which is therefore a kind of parthenogenesis. The failure of species crosses such as these to produce normal embryos when normal sperm is used is therefore due to the incompatibility of the nuclear materials of egg and sperm. O. Hertwig (1913) obtained embryos from *Triton* eggs fertilised with irradiated sperm of *Triton* and of *Salamandra*. P. Hertwig (1916, 1924) subjected the eggs of *Triton* to radiation and fertilised them with normal sperm and obtained embryos.

The haploid nature of these embryos was proved by counting their chromosomes and measuring their nuclei. Haploid tissue can therefore always be recognised, and G. Hertwig (1925) has made use of this fact to recognise and identify grafts and the structures to which they give rise apart from the tissues of the host.

It is worth mentioning that Lewy (1913) was able to rear embryos of *Rana esculenta* and *temporaria* (which had been activated by Bataillon's (1904) pricking method) past the stage of metamorphosis into little frogs.

4. DETERMINATION DURING GASTRULATION.

Spemann (1903) observed that when embryos of *Triton* were divided or constricted in the sagittal plane in the blastula and up to the early gastrula stages, normal twin embryos and duplicitas anterior depending on the degree of constriction could be obtained. At later stages however no such regulation of the parts took place. The time of determination must therefore lie somewhere between the beginning and end of gastrulation. Additional evidence is obtained from cases of twinning in other animals. Stockard (1921) exposed late cleavage stages of *Fundulus* to low temperatures (5° C.), and in other experiments deprived them of oxygen. The result was twins and double monsters. After gastrulation is completed however, the development can be stopped or inhibited with comparative impunity. Newman (1923) as a result of study of twinning in the armadillo concludes that the gastrula is the critical stage. Lebedinsky (1923) correlates the frequency of twinning in Teleosts, Reptiles, Birds and Mammals with their blastoderms and flat gastrulae; as contrasted with the Cyclostomes, "Ganoids" and Amphibia in which the gastrula is spherical and twinning is infrequent. The point is that no twinning or regulation takes place after gastrulation has been completed.

Spemann tested the determination of different regions of the newt at different stages by means of transplantations (1918). In the following descriptions the word

"presumptive" is used to mean the prospective fate of any given piece of tissue in normal development. At the beginning of gastrulation a piece of presumptive epidermis (from the flank or ventral region) was exchanged for a piece of presumptive nerve tube. The pieces differentiated according to their new surroundings and regardless of their origin. This proves that at this stage they are not irrevocably determined. It is interesting to notice that a ventral piece can become nerve tube, although a ventral half-gastrula deprived of the dorsal lip region will not differentiate. What the ventral half lacks therefore is not potency but a factor, which will be considered in the next section.

The transplantation experiment can be made still more striking by using different species or genera as "donors" and "hosts." The tissues of *Triton cristatus* are light and free from pigment, those of *Triton taeniatus* are dark; they can therefore readily be distinguished (Spemann, 1921). A piece of presumptive *cristatus* epidermis in *taeniatus* anterior nerve-tube region will differentiate into an eye cup. A similar piece in the otic region of a *taeniatus* embryo will develop into a proper ear. *Bombinator* presumptive epidermis grafted into the anterior nerve-tube region of *Rana esculenta* will give rise to an eye cup, the lens corresponding to it being formed of *esculenta* tissue. A very interesting case is that in which a piece of *taeniatus* presumptive nerve-tube region is grafted on to the flank of a *cristatus* embryo. The graft differentiates into gills according to its new position, but it preserves its *taeniatus* character in that these gills are larger and better developed than the normal *cristatus* gills on the other side of the embryo. This difference at the same stage is observable between the two species. In other cases (Spemann, 1918) in which a graft of presumptive epidermis from a younger donor is grafted into the anterior nerve-tube region of an older host, the graft differentiates into an eye cup. This eye cup is smaller than the normal other one of the host, but is similar in size to the remaining one in the donor. While therefore there is no determination at this early gastrula stage, the tissues nevertheless preserve some of their original peculiarities.

An exchange between presumptive epidermis and nerve-tube pieces in an older gastrula of *Triton*, 21 hours before the neural folds appear (room temperature), leads as before to differentiation according to the new position of the pieces, only this time less completely. If the exchange is performed on a still older gastrula, when the blastopore is a slit and 9 hours before the neural folds appear, the pieces differentiate according to their place of origin, and thereby show that they have been irrevocably determined at this stage. Determination in respect of the nerve cord therefore sets in in *Triton* towards the end of gastrulation.

Confirmation of this is found in the power of regulation at different stages of constricted dorsal half-gastrulae of *Triton*, studied by Ruud and Spemann (1923). These dorsal halves contain the dorsal lip of the blastopore and consequently are capable of development. Should they be constricted off at the early gastrula stage, the resulting embryo is perfect and has a properly proportioned nerve tube for its size, although it contains the material which in normal development goes to make a nerve tube double this size. At this stage therefore the dorsal half-gastrula is

still capable of regulation, and the nerve tube is not yet determined. If the dorsal half is constricted off at a later stage when the blastopore is small and oval, yet still open, regulation no longer takes place and the resulting embryo develops with neural folds which are relatively much too large for it. It is worth noticing that whereas the power of regulation ceases at this stage with small, oval yet open blastopore, transplantation experiments prove that the definite determination of the neural tube does not take place until a slightly later stage, when the blastopore is closed and slit-like. This slight difference in time is to be expected and means that the processes of regulation themselves take a certain time. If they are initiated too late, the structures will be determined before the processes have power to act. If the time discrepancy were the other way, it would be inexplicable.

In *Fundulus*, Lewis (1912) found that determination had taken place at the comparable embryonic shield stage. An injury to the posterior end of the shield prevented the formation of the tail and hinder region of the body; an injury at the anterior end prevented the formation of the head.

In newts and *Fundulus*, therefore, the parts of the embryo cease to be equipotential, and the neural tube is determined at a late gastrula stage.

Before leaving this subject, attention must be paid to some other interchanges of regions before determination sets in. By means of *intra vitam* reference marks, Goerttler (1925, 1926) has shown which regions of the blastula (when the dorsal lip of the blastopore is just appearing) are to be regarded as presumptive ectoderm, presumptive notochord, presumptive endoderm and presumptive mesoderm. Mangold (1924) has carried out extensive potency tests on these regions at the blastula and early gastrula stages. He found that a piece of presumptive ectoderm could become part of notochord, somite, pronephros, splanchnopleur, somatopleur and gut wall, according to the position it reached during gastrulation, which again depended on the time and place where it was grafted. The first four possibilities enumerated above followed the implantation in the blastopore lip, the last resulted from placing in the blastocoel. Presumptive mesoderm can be made to form ectoderm. Presumptive endoderm does not do well in an ectodermic region owing to its passivity and tendency to become overgrown. For although the various regions at this stage are not restricted in their potency, yet they are distinguished by tendencies which lead to the normal process of gastrulation: growth and extension of surface of the animal pole region, growth and inflexion of the blastopore rim and passivity of the vegetative pole region. Transplanted portions "behave" as they would have done if left alone, but they differentiate according to their new positions. It is interesting to compare this phenomenon with those already mentioned as regards retention of certain specific and age characteristics (size of eye and gill in *Triton*) and with the cases among Invertebrates of isolated blastomeres cleaving as parts and developing as wholes. After the yolk-plug stage is reached, these mass-movement tendencies disappear. For the rest, these implants tend to adapt their cell size and division speed to their surroundings, but not always; sometimes the grafts grow much more rapidly than their surroundings. It may be added that the recognition of the implants in the various situations was guaranteed by the heteroplastic method (*Triton taeniatus* and *cristatus*).

5. THE ORGANISER.

During the experiments just described dealing with the determination of the neural tube at the gastrula stage in *Triton*, Spemann (1916) observed that in some cases while the presumptive neural tube material in an anterior region might still be indifferent, that at the hinder end near the dorsal lip of the blastopore was already determined. This looked as if a "flow of determination" started from a definite place, the dorsal lip of the blastopore, and spread thence over the embryo. This suspicion is further supported by the fact that if in the early gastrula stage of *Triton* the animal hemisphere be cut off, rotated through 90° or 180° about the egg axis and stuck on again, the neural folds arise in a line in front of the dorsal lip of the blastopore (situated in the ventral hemisphere) from material which would normally never have formed them. At the same time, from the original presumptive neural fold material which has been rotated away, neural folds do not form (Spemann, 1906*b*, 1918). Further, if two gastrulae are divided sagittally and the two right halves stuck together on the one hand and the two left on the other, each composite embryo has two half-blastopores, one on each side. Each completes itself into a whole blastopore with the result that double monsters are formed from each embryo. The determination and differentiation of the embryo therefore appear to be dependent on the dorsal lip of the blastopore. This was proved beyond doubt by Spemann and Mangold (1924), who transplanted a piece of the dorsal lip of the blastopore of one embryo into the flank of another at the gastrula stage. Here the implant induced the formation of a second embryo with nerve tube, auditory vesicles, notochord, somites and pronephros. Some of the structures of this secondary embryo were formed from the host's tissue, and some from the graft. The notochord was always formed from the graft, the nerve tube mostly from the host cells, somites could be formed from either alone or both together. In some of Bautzmann's (1926) experiments the secondary embryo was as perfectly formed as the primary. From this power which the dorsal lip of the blastopore possesses of organising the main structures of an embryo, it has been given the name of *Organiser*.

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Fig. 4. Left side view of an embryo of *Triton taeniatus* into which an organiser has been grafted. The figure shows the dorsal side of a well-formed secondary embryo. Note that the auditory vesicles of the primary and secondary embryo are at the same level. (From Spemann.)

Geinitz (1925 *a*) carried this astounding analysis further when he showed that the organiser could work not only in grafts between different species and genera, but also between subclasses. Organisers of *Pleurodeles waltli* and of *Amblystoma mexicanum* induced secondary embryos in *Triton taeniatus*. When an organiser from *Bombinator pachypus* was grafted into *Triton taeniatus*, a neural tube, auditory vesicles and somites were induced from the tissues of the host, and at the same time the graft itself differentiated into another nerve tube, a notochord and mesenchyme. The reason for this duplicity of the nerve cord is that there is too much incom-

patibility between the tissues of *Bombinator* and *Triton* to contribute together to form one organ. While in the organiser grafts between *Triton cristatus* and *taeniatus* both took part in the formation of a single nerve tube, in the case in question each tissue differentiated by itself since "chimaeras" cannot be formed. This case is further interesting as showing that the action of the organiser is neither by self-differentiation nor by induction alone.

It should be noted that a notochord (which always arises from the graft) is only formed when the organiser is taken from the centre of the dorsal lip of the blastopore. When taken from the side no notochord is formed though a nerve tube is induced. *Rana temporaria* and *esculenta* organisers also induce embryos in *Triton*. The converse grafts do not succeed because of the difficulty of freeing the gastrulae of *Anura* from their vitelline membranes.

The complete gastrula is the last stage from which a functional organiser can be obtained.

It must not be forgotten that while the detailed analysis of these phenomena is the work of Spemann and his school, the credit of making the first organiser graft belongs to Lewis (1907 *b*). He grafted the organiser from a gastrula of *Rana palustris* into the otic region of another embryo in the tail-bud stage, and obtained an induction of nerve tube, notochord and somites.

Brachet (1923) further investigated the properties of the organiser in *Anura*. Working on *Rana fusca* in the late blastula stage, he found that injuries (made with a needle) just below the grey crescent resulted in embryos nearly normal, while if they were above the grey crescent, the head was deficient and showed asymmetry. The malformation is however quantitative, not qualitative, the brain and neural crest are morphologically normal but too small in one region. If injuries are made in a lateral horn of the grey crescent, the trunk on that side is abnormal as regards nerve tube, notochord and somites. An interesting point to notice is that while these organs are rudimentary owing to lack of material in the injured region, in the (supposedly) uninjured region immediately behind it, they continue to be of the same abnormally small size. To explain this Brachet supposes that a formative impulse travels in a cranio-caudal direction, and that this impulse is too weak in the injured region to induce any but similarly rudimentary organs in the region immediately behind it. But this view is perhaps unnecessary when it is realised that the sides of the grey crescent (and blastopore lip) are constantly growing down and producing new material. One would expect therefore that an injury there would affect all regions posterior to it.

Injuries to the centre of the grey crescent result in large deficiencies as regards nerve tube, notochord and somites. Here also the notochord-forming zone appears to be restricted to the centre of the grey crescent.

Brachet concludes that the grey crescent is a region of "spontaneous" differentiation. "Spontaneity" merely means that the antecedent causes are unknown, except that in this case they must be connected with the entrance of the sperm.

Brachet's and Spemann's results are on the whole as similar as could be expected, remembering the difference between the subclasses *Urodela* and *Anura*. The differences seem to be due to three factors (or groups of factors):

(i) Difference of experimental method, as shown by the fact that when an anuran organiser is transplanted and grafted into other *Anura* (Lewis, 1907 *b*) or into *Urodeles* (Geinitz, 1925 *a*), the results are very similar to those obtained by grafting urodele organisers into *Urodeles* (Spemann and Mangold, 1924). Injury with a needle is not as precise a method as transplanting with a micropipette, since it is difficult to be sure of the extent of the injury.

(ii) Brachet (1923) believes that in *Anura* only the anterior region of the head is preblastoporal, while in the *Urodeles* the head and trunk are preblastoporal. Goerttler's (1926) *intra vitam* markings show that the animal pole of the newt's egg becomes included behind the transverse anterior neural ridge, while that of the frog lies just in front of it. Further, in *Anura* the dorsal lip of the blastopore arises much nearer the equator than in *Urodeles*. However, the behaviour of anuran organisers in urodele tissues shows that the difference in this respect is not fundamental.

(iii) The various regions are possibly determined earlier in the frog, some at least soon after fertilisation; in the newt towards the close of gastrulation. The early specialised appearance of the presumptive organiser (grey crescent) in frogs is perhaps correlated with this accelerated *tempo* of determination. In this connection it is interesting to note that Bautzmann (1926) obtained an organiser from the early blastula stage of *Triton*.

These facts concerning the organiser immediately reveal the importance and significance of the experiments of isolating blastomeres in the 2-cell stage when it was found that only those blastomeres which contain the future dorsal lip of the blastopore would develop. This is also the explanation of the failure to develop of the ventral gastrula halves of *Triton*. It has already been mentioned that what these lack is not potency but a stimulus: the organiser. That this is so has been proved by Bautzmann (1926), who grafted an organiser into a ventral half-gastrula, and obtained the induction of an embryo.

6. ANALYSIS OF THE METHOD OF FUNCTIONING OF THE ORGANISER.

In order to explain the results which the organiser achieves, Spemann and Mangold (1924) put forward two suggestions. The effect may be due to an impulse travelling from cell to cell radiating out from the region of the organiser (a "differentiating stream"), or it may be due to the contact of the invaginated roof of the archenteron with the overlying ectoderm.

There is more than one example of supposed streams of differentiation. Braus (1906 *a*) shows something similar for the fins of sharks, and there are several examples of neuroid transmission.

Supporting the second alternative is an observation of Vogt (1922 *a*) to the effect that those embryos which lacked neural folds also lacked the roof of the gut. Geinitz (1925 *a*) removed a piece from a late gastrula of *Triton* close in front of the dorsal lip of the blastopore, and divided it tangentially into an outer (non-invaginated) and an inner (invaginated) portion which he then grafted into new hosts. He found that both portions induced a nerve tube and that in addition the former

differentiated into nerve tube and notochord, the latter differentiated into a notochord and mesenchyme.

Marx (1925) working on *Triton* in the late gastrula stage showed that if a piece of presumptive nerve tube were transplanted to the flank *without* any underlying gut roof being taken with it, it differentiated according to its new position into epidermis. If on the other hand some gut roof was included in the graft, it differentiated into neural folds. From earlier embryos, presumptive neural tube material always develops according to its new position whether gut roof is present or not. From older embryos, presumptive neural tube material, whether gut roof is included or not, is always self-differentiating. The only conclusion to draw is that the neural tube is determined at the late gastrula stage when the gut roof has invaginated and is intimately apposed to the underside of the presumptive nerve-tube material. Marx also calls attention to the remarkable correlation and correspondence in size which exists between the neural plate and the archenteron in embryos of several groups of Vertebrates.

This is supported by Geinitz' (1925 *a*) observation that *Bombinator* gut roof in *Triton* induces neural folds, as does *Triton* gut roof in *Triton* (Marx, 1925). Bautzmann (1926) found that at the yolk-plug stage in *Triton*, the hinder two-thirds of the gut roof in the median and paramedian region had this capacity. He also found that, at the outset of gastrulation, the zone which was capable of functioning as an organiser coincided with that which Goerttler (1925) showed to undergo future invagination. Lehmann (1926) tested the inducing power of the gut roof by practising deficiencies at the edge of the lip of the blastopore at various stages of gastrulation. Removal at an early stage results in deficiency of the anterior gut roof and mesoderm. The overlying neural folds show a lack of lateral ridges, too thick a floor and an atypical orientation of nuclei. The neural folds are most atypical where the underlying gut roof and mesoderm is most deficient, but the correspondence is not so exact at the front end. The notochord is often absent and in such cases the somites fuse in the middle line; the nerve tube then shows a peculiar abnormality in that the floor is much too large. He concludes that the final form of the nerve tube is influenced quantitatively by the deficiency in the underlying material.

There can be little doubt after consideration of this evidence that the organiser works by pushing itself underneath the epidermis of the dorsal region and affecting it in some way. (While the nerve tube certainly is formed in this way, it will be necessary further on to consider whether it may not also have been determined before gastrulation by another means.) It explains why presumptive nerve-tube material near the blastopore is irrevocably determined sooner than that further forward, for the undergrowth takes place from behind forwards (Spemann, 1916). It explains why dorsal half-gastrulae regulate the size of their neural folds if isolated early enough (Ruud and Spemann, 1923). It explains why, by partial constriction of *Triton* embryos in the sagittal plane, Spemann (1903) obtained duplicitas anterior, since owing to the ligature the invaginated gut-roof material had to divide itself right and left and thereby induced two embryos in the anterior region; also why he never obtained duplicitas posterior by this method, since there was room

for the hinder gut roof (the "last-to-be-invaginated" material) without any necessity for it to split. Lastly it explains certain remarkable cases of duplicitas cruciata which Wessel (1926) has studied and described.

If some of the preblastoporal region of two young *Triton* gastrulae be cut off and the two gastrulae stuck together by the cut surfaces so that the blastopores gastrulate towards one another, the result varies with the distance which separates the dorsal lips of the two blastopores. If this is large the two embryos will form parallel to and facing one another on each side of a common yolk mass. The median ventral organs such as the heart and liver are then formed at the sides, and from half-components of each embryo which can be distinguished by pigment. If the distance separating the blastopores is smaller, the head ends of the two embryos will meet and turn out right and left, so that the combined neural folds form a cross. Two secondary head ends are formed in this way, each composed of tissue from each embryo. If the distance is still further decreased, the clash comes very soon, and most of the embryos are "secondary" and composite, at right angles to the original axis, and only the tails are "primary." Several points are worth noticing here: (i) formation of heads and trunks of embryos from "non-presumptive" material; (ii) alteration of axes and polarity; (iii) formation of organs at the right "level" though not in the normal axis from components of two embryos. The lack of room between the sites of invagination leads to a collision and the nerve folds deviate right and left. But the nerve folds do not arise by any process of growing forward, they arise *in situ*, and what do grow forward, collide and move to the side, are the underlying ingrowing gut roofs. This only can explain the formation of a duplicitas cruciata. Bautzmann (1926) also obtained duplicitas cruciata by organiser grafts.

In the induction of an embryo by an organiser, it is of interest to enquire into the part played by the host into which it is grafted, and the first problem which presents itself is that of orientation. Although the axis of the secondary embryo may make some angle with that of the primary host embryo, yet they never form in reversed directions, head to tail. By grafting organisers of triangular shape, and orientating them sideways or backwards with regard to the axis of the host, Geinitz (1925 *b*) found that the secondary embryo was always more or less parallel to the primary one. This points to an axial directive effect of the host tissues and of the embryo in general. Further, with regard to the position of corresponding organs in the primary and secondary embryos, it is of great interest to note that they tend to arise at the same level with regard to the animal pole, *i.e.* on the same parallel of latitude. Especially is this the case with the auditory vesicles (Spemann and Mangold, 1924; Bautzmann, 1926).

Lastly, there is the question as to what constitutes the difference between the organiser and other common pieces of tissue, in virtue of which their properties and fates differ. It is premature to speculate too much on this problem, but Geinitz (1925 *c*) has obtained some facts which furnish much food for thought. He took a piece of ordinary presumptive epidermis and grafted it into the region of the organiser so that it was carried in with the invagination; for purposes of recognition it was stained *intra vitam* or obtained from a different species. When it had been

invaginated and formed part of the gut roof it was removed and transplanted a second time into the side of another embryo, and here it proceeded to function as an organiser. This piece of ordinary epidermis had therefore been "infected" with the power to organise during its sojourn in the organiser region. This shows that the properties of the organiser are not tissue-specific in the first instance, but that they are determined by the relative position with regard to the whole embryo. This will form a subject of discussion in the section on Axial Gradients.

7. DETERMINATION AND DIFFERENTIATION IN THE BLASTODERM OF THE CHICK.

The behaviour of different regions of the chick's blastoderm at various stages of development has been tested by grafting pieces on to vascular regions of the chorio-allantoic membrane of 7-day-incubated chicks. Attempts had been previously made to obtain development *in vitro*, by McWhorter and Whipple (1912).

With *intra vitam* staining marks Wetzel (1925) showed that tissue at the posterior edge of the blastoderm stretches forward in the formation of the primitive streak. Peebles (1898) showed by means of injuries with a needle that in later development the primitive streak pushes backwards.

Danchakoff (1922) observed that the less a blastoderm has been incubated, the less growth and differentiation is obtained when it is grafted entire on to the membrane. From the advanced primitive streak stage onward, good differentiation is obtained. Kidneys, eyes, nervous system and notochord develop well.

Hoadley (1926 *a*) has made a close study of the powers of differentiation of isolated portions of blastoderms at different stages of development. Portions of the unincubated blastoderm will differentiate only into gut and epidermis. After 2 hours' incubation a graft will also produce the nervous system. After 4 hours, brain, eye, cartilage and muscle appear. A piece of blastoderm which has had 6 hours' incubation will after grafting show in addition ganglia, fibres, ear, glands, somites, notochord, mesonephros and heart. After 10 hours' incubation, corium and feather buds are formed. Thus it is plain that the older a blastoderm or portion of it is at the time of grafting, the more will it differentiate. This is especially well shown in the case of the eye, by grafting transverse strips of blastoderm containing the presumptive eye material. A 4-hour piece will give an eye of pigment cells only. A 6-hour piece will show differentiation into pigment and retinal cells. After 8 hours, differentiation goes as far as to show stratification of the retina. Complete self-differentiation of the eye is obtained from pieces of blastoderms which have been incubated for 33 hours. Similarly, with regard to feather buds (Hoadley, 1926 *b*), pieces from a blastoderm incubated for less than 10 hours will only produce the periderm and columnar layers; after 10 hours corion will be produced. In the case of the mesonephros, 4-hour pieces will give secretory tubules, 6-hour pieces also produce glomeruli, 10-hour pieces give Wolffian ducts, and if the pieces have been incubated for more than 10 hours, the entire mesonephros is differentiated (Hoadley, 1926 *c*). He concludes that these experiments are evidence for the existence of "preprimordial segregates," and that there is "progressive differential

dichotomy." Isolation, however, prevents further dichotomy without preventing the histogenesis and differentiation of the elements already determined by previous dichotomies. Hoadley (1926 *d*) has amplified these results by cutting across a blastoderm and leaving it to develop *in situ*. Here again the isolation interferes with the further morphological differentiation, without impeding the progress of the histological differentiation already reached. It is plain that the pieces are not self-differentiating until a certain stage of development has been reached; which means that they are dependent for their further differentiation on a factor situated outside them. It is tempting to compare these results with those of Hyman (1916) on the regeneration of heads from the posterior regions of the worm *Lumbriculus*. The differentiation of the regenerate into a head is governed by the size of the piece regenerating, and it is possible that a comparable state of affairs exists here. Indeed it is very probable, for Hoadley (1925 *b*) found that in the differentiation of the feather primordia, the larger the piece of blastoderm grafted, the less was the inhibition of the differential dichotomy, *i.e.* the more differentiation was obtained. He admits that if an *entire* blastoderm were grafted, differentiation would be complete.

From pieces of blastoderm that have been incubated for 36 to 48 hours, the rudiments of the eyes, ears and nose will develop by self-differentiation as grafts (Hoadley, 1924). Similarly, after 36 hours' incubation, somites, pronephros and neural crest (Hoadley, 1925 *a*). Agassiz and Danchakoff (1922) showed that, provided it is closed at the time of grafting, the neural tube will differentiate into a piece of spinal cord with grey and white matter, horns and nerves. From 7-day chicks a graft of the metanephric rudiment differentiates completely, capillaries of the allantois being induced to form glomerular knots in the concavities of the Bowman's capsules (Atterbury, 1923). Rienhoff (1922) cultivated the rudiment of the metanephros *in vitro* and obtained complete development, tubules and glomeruli differentiated *in situ*. From the 7-day chick Danchakoff (1924) found that the rudiments of the following organs would differentiate: spleen, pancreas, hypophysis, liver, kidney, adrenal, thymus, thyroid, muscle, nerve tube, heart, ovary and testis. Periosteum develops bone. She also made the very interesting observation that the mesonephros degenerates after a time in the grafts, as indeed it does in the organism.

Danchakoff (1922) grafted the anterior half of a blastoderm at the 10-somite stage, with the curious result that the pronephros was better developed than in a normal embryo. This is probably due to the removal of an inhibiting influence from the more posterior kidney elements.

Using larger pieces, Murray and Huxley (1925 *a*) showed that the anterior third of a 24-hour blastoderm would differentiate into a complete anterior region. The brain had the proper subdivisions, epiphysis, eyes, pituitary, lens, otic sac, grey and white matter, cranial nerves, olfactory pits, mouth, pharynx, anterior intestinal portal, thyroid, and visceral pouches were all present. It is interesting that the bulbus and ventricle only of the heart were formed. Presumably at the time of grafting the various regions of the heart were determined, and the cut

separated these from the auricle and sinus rudiments. From the middle region of a 48-hour blastoderm, spinal cord, dorsal and ventral nerve roots, vertebrae and mesonephros were formed. While there was little abnormality in the histological differentiation of the parts, their shape was often abnormal owing to the peculiar conditions of pressure and stress to which the embryo was exposed on the chorio-allantoic membrane. This shows the importance of the fact that the determination of an organ and of its future histological differentiation (which is quantitative and chemical) is to be distinguished from the processes which lead to morphological differentiation (which are largely mechanical and physical, and governed by conditions of pressure and available space).

Lastly it must be mentioned that Strangeways and Fell (1926 *a*, *b*) cultivated rudiments of eyes and limbs *in vitro*; chorio-allantoic grafts of limbs were also studied by Murray and Huxley (1925 *b*) and Murray (1926); the detailed descriptions of these experiments are reserved for the special sections dealing with these organs.

This perhaps is the place in which to mention some remarkable experiments of Brachet (1912, 1913) in which he cultivated young blastodiscs of the rabbit in serum *in vitro*. A blastodisc aged 5½ days doubled its diameter in 24 hours. After 48 hours it showed the primitive streak and chorionic villi. A blastodisc 6¼ days old developed amniotic folds after 24 hours, and the rudiment of the notochord was plainly visible after 44 hours. In one case, an accidental tear went through the primitive streak, and it is very interesting to note that in this case the notochord and mesoderm failed to differentiate. The self-differentiation of the villi is remarkable, showing that they are not dependent on the wall of the uterus.

8. THE NERVOUS SYSTEM.

In the late blastula stage of *Urodeles*, Goerttler (1925) has shown that the presumptive neural fold material is situated in a transverse narrow zone of tissue stretching across the animal hemisphere, including the animal pole and reaching down to near the equator on each side. As a result of the movements which bring about gastrulation, the side portions of this zone come to be directed backwards and are stretched along the future antero-posterior axis. The result is to bring the presumptive neural fold material into the position which these folds occupy in the neurula.

Sufficient has been said in previous chapters to show that (*a*) the presumptive neural fold material is not irrevocably determined until the late gastrula stage, and that (*b*) the organiser has the power of determining neural folds out of non-presumptive neural fold material. These facts do not however exclude the possibility that the neural folds may be determined before the late gastrula stage, a possibility expressly considered by Spemann (1918). Goerttler (1925) has tried to test this by preventing the displacement of the material from proceeding normally. This he did by making injuries or grafting foreign pieces into the region of the lateral lip of the blastopore of embryos of *Pleurodeles*. The result was that the presumptive neural fold material did not reach its normal destination. Yet the folds actually

formed from the presumptive material and were therefore out of place. This points to independent determination of the neural folds as regards the organiser, which, in other cases is able to induce normal neural folds. Further, Goerttler found that removal of a piece of presumptive neural fold material at the beginning of gastrulation resulted in permanent deficiency as regards the neural fold in that region, although the wound had been healed over. One would expect that as neural fold material is not irrevocably determined at this stage, and further that as the organiser can induce neural folds, the deficiency would be made good. These results are therefore in contradiction with those of Spemann and his school. Lastly, Goerttler

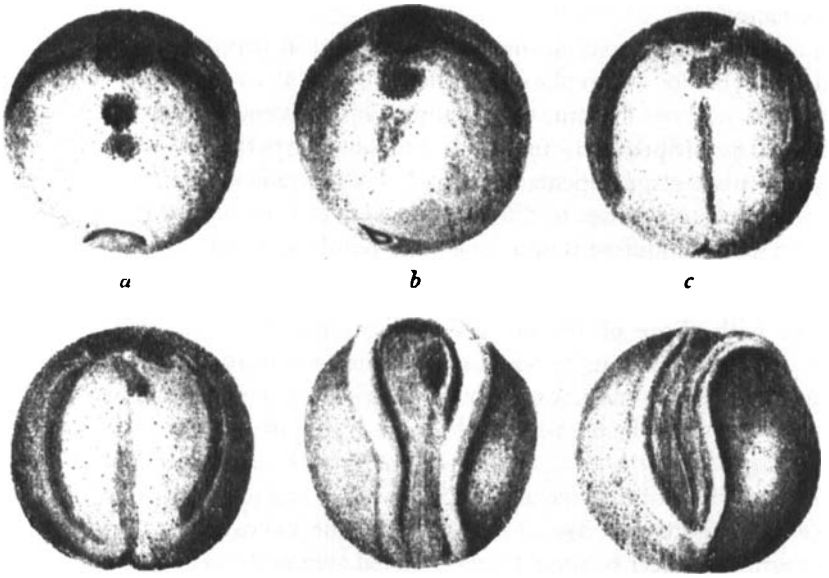

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Fig. 5. Embryo of *Triton alpestris* with *intra vitam* marks in the presumptive neural fold material. Note the displacement of the lateral bands which become included within the folds, and the stretching of the tissue in the mid-dorsal line. (From Goerttler.)

(1926) removed the whole region of the dorsal lip of the blastopore at its earliest appearance in *Pleurodeles* embryos. Nevertheless, the neural folds arose from the presumptive material, which had been marked *intra vitam*. He concludes that the organiser and gut roof play no essential part in the determination of the neural folds. (It must be remembered that Lehmann (1926) found the front end of the nerve tube somewhat independent of the gut roof.) Further, Dürken (1925 *b*, 1926) obtained differentiation of a portion of a brain from a piece of the animal-pole region of a blastula of *Rana fusca*, *i.e.* a piece which had not been acted on by an organiser. This being so, the neural folds must be a structure capable of self-differentiation and also of dependent differentiation at the same time. In the course of this study several other cases will be met with of organs developing by the method of "double assurance," though in almost every case the self-differentiating property can be shown to set in after the dependent differentiating processes. In any case,

self-differentiation is at best a negative conception since it means only that at the time when it is tested, no correlation can be discovered between the structure in question and neighbouring structures. In the case of the neural folds, therefore, the possibility is open that the organiser determines them long before the dorsal lip of the blastopore appears, though the determination is not irrevocable until the late gastrula stage. In this connection it may be remembered that in the frog the organiser is foreshadowed as a very early differentiation in the form of the grey crescent, while in *Urodeles* it is not nearly so definite. This seems to be the only way of reconciling these apparently contradictory results, *i.e.* by appealing to the time factor.

If a rectangular piece is cut out of the mid-dorsal region of a gastrula of *Triton*, rotated through 180° and replaced (*i.e.* head to tail), the resulting embryo is normal as regards its nervous system, only it shows situs inversus viscerum, which will be considered later (Spemann, 1918). If the same experiment is performed at the open neural plate stage (Spemann, 1912 *b*) the nervous system is abnormal in that the rotated piece continues to differentiate as if it were still in its normal position, *i.e.* it is no longer indifferent and capable of regulation, but is determined. In such embryos the optic lobes are in front of the epiphysis and diencephalon, and the self-differentiation of regions is apparent even close up to the cut edges which have healed up with those of the surrounding normal tissue (experiments on *Rana esculenta*). (It is interesting to note in connection with the closure of the nerve tube that Giersberg (1924) has shown that it is in part a mechanical process, since the repartition of mitotic figures by itself is incapable of accounting for it. There is lateral pressure from the ectoderm as well as growth of the neural fold.)

The spinal cord of *Amblystoma* has been subjected to a very interesting analysis by Detwiler. From the medulla backwards the nerve tube tapers, so that the various segments differ in their cross-sectional area and in the number of cells and fibres they contain. If trunk segments 7, 8 and 9 of the tube (in tail-bud-stage embryos) be transplanted to the position of segments 3, 4 and 5, they attain the proper size for their new region (Detwiler, 1923 *a*). Similarly if 3, 4 and 5 be removed and replaced as 5, 4 and 3 (Detwiler, 1923 *b*), the original 5 behaves as if it were a normal 3 and the original 3 shows a reduction to the size of a normal 5. In this case the nerves of the brachial plexus were normal also. The next experiment was to remove segments 1, 2, 3, 4 and 5 and to replace them with a medulla and segments 1 and 2 from another embryo, in the proper orientation (Detwiler, 1925 *a*). The medulla occupied the regions of segments 1, 2 and 3, transplanted segments 1 and 2 were in the position of 4 and 5. It was found that these segments 1 and 2 contained more cells in the motor regions than if they had remained in their normal position. The reason for their containing more cells than normal is to be found in the increased number of impulses reaching them from the brain, which increase is itself due to the increased number of caudal projection fibres arising from the extra medulla. To prove this, segments 1, 2 and 3 were transferred to the region of 4, 5 and 6 (Detwiler, 1925 *b*). By this means 1 and 2 were in the same position as in the previous experiment, only there was no extra medulla. The

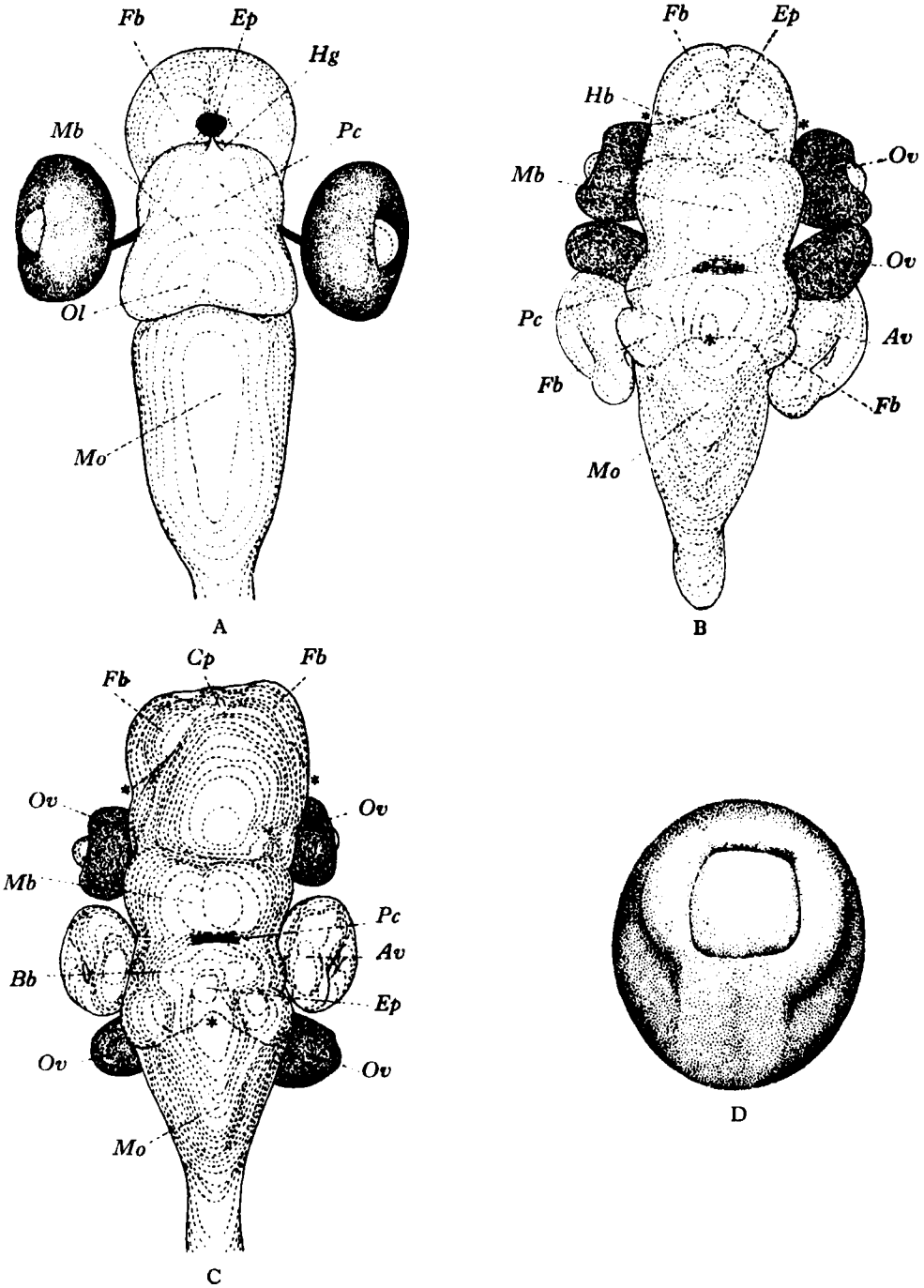

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Fig. 6. A. Dorsal view of the brain of a normal embryo of *Rana esculenta*. B. Similar view of an embryo in which a short piece of the medullary plate (between the asterisks) has been rotated through 180°. Note 4 small optic vesicles. C. Similar view, after rotation of a larger piece of the medullary plate. Note 4 optic vesicles; the posterior pair behind the auditory vesicles. D. Embryo of *Rana esculenta* at the neurula stage showing the piece of the medullary plate which is rotated in these experiments.

Av, auditory vesicles; *Bb*, between-brain; *Cp*, choroid plexus; *Ep*, epiphysis; *Fb*, fore-brain; *Hb*, hind-brain; *Hg*, habenular ganglion; *Mb*, mid-brain; *Mo*, medulla oblongata; *Ol*, optic lobe; *Ov*, optic vesicle; *Pc*, posterior commissure. (From Spemann.)

result was that segments 1 and 2 maintained their normal size, which is therefore determined and self-differentiating. For the remaining segments the degree of motor cellular proliferation appears to be commensurate with the number of projection fibres. It is interesting to note in these experiments that while the nervous system is early determined as a whole, up to a certain stage it retains the power of alteration within itself, *i.e.* it is capable of regulation. After that stage it loses this power. Burr (1920) showed that at a certain stage the cerebral hemispheres of *Amblystoma* are self-differentiating, by grafting them behind the fore limb. They differentiated more or less normally, though with distortions, and reduced size owing to the lack of ascending fibre tracts.

In the newt the (motor) cellular proliferation in the nerve tube is not determined by functional activity of the peripheral musculature (Detwiler, 1924). This is shown by the fact that transplantation or removal of a limb does not affect it. On the other hand the (sensory) spinal ganglia are greatly affected by increasing or decreasing the peripheral integumentary areas. Detwiler (1926) grafted two *Amblystoma* embryos together side by side with the result that the skin area, and consequently also the spinal ganglia on the inside were much reduced. Shorey (1909) however found that in *Bufo*, *Rana pipiens* and chick, loss of a limb entailed deficiencies in the spinal cord as well as in the spinal ganglia.

Steinitz (1906) removed the eye from a frog embryo and observed a deficiency in the optic lobes. Dürken (1913, 1917) working on *Rana fusca* found that if the eye were extirpated in very early stages both the optic lobes were deficient; and the retina of the other eye was subnormal in its differentiation. If the experiment was performed later only the contra-lateral optic lobe was affected. Burr (1924) observed that after implantation of an extra olfactory organ in *Amblystoma* close to the host's own nose, there was a cellular increase of 30 per cent. in the olfactory bulb. May and Detwiler (1926), also using *Amblystoma*, grafted an extra optic vesicle behind the ear. When the optic nerve from the graft established connection with the glossopharyngeal or vagus ganglia, these showed cellular increase, and the medulla oblongata was normal. If the optic nerve entered the medulla direct, then it showed hyperplasia in its grey matter. If at an early stage a limb rudiment is removed, all the centres in the nervous system associated with limbs are underdeveloped (Dürken, 1912). When this experiment is performed at a later stage, if the fore limb was removed the contra-lateral centre in the mid-brain was deficient, if the hind limb, the centre on the same side. These results must be due to general debility following on lack of nervous correlation and stimulation. These experiments will be mentioned again in connection with certain peculiarities which the limbs show.

The ganglia of the cranial nerves are derived from two sources: the neural crest, and the placodes (areas of thickened epidermis). Stone (1922) attempted to show that in *Amblystoma* the cranial ganglia were largely dependent on placodes for their formation. Extirpation of the ophthalmic placode led to absence of ophthalmic ganglion and nerve. Removal of the gasserian placode resulted in deficiencies in the gasserian ganglion, and similarly in respect of the hyomandibular placode and

the facial ganglion. It is possible however that neural crest cells may also have been removed with the placode. In another experiment (Stone, 1924) the ophthalmic placode of one embryo was grafted close above the normal similar placode of another embryo. The graft gave rise to a ganglion from which fibres developed and innervated regions normally supplied by the host's own ganglion. Further, when the ophthalmic placode is grafted in the place of an extirpated gasserian placode, the ganglion so formed partly replaces the gasserian in form and nerve distribution, in particular, contributing to the mandibular nerve.

These experiments show that a certain amount of regulation takes place in nerve grafts but that their histological (chemical) differentiation is already determined. This is to be expected from Harrison's (1910) remarkable tissue cultures of nerve cells from embryos of *Rana palustris*. The cells produced axon fibres which grew out (in one case) at the rate of 56μ per hour. The longest axon thus obtained reached 1·15 mm., having taken 53 hours to grow. Ingvar (1919) carried the analysis a stage further by showing the reaction of these axons to electric currents. The fibres grow out along the lines of the field of force of a very weak current. If however a conductor goes through the culture and a current passes through it, the fibres grow out at right angles to this conductor. Now it has been shown in many animals (see Hyman and Bellamy, 1922, and Child, 1924) that a difference of potential exists between the anterior and posterior ends of bilaterally symmetrical animals. Consequently it can be understood why fibres grow up and down the nerve tube and also, since when impulses travel through them these fibres act as conductors, why other fibres then grow out at right angles to them, as do the spinal nerves.

This is perhaps the place in which to consider the question of the outgrowth of nerve fibres to their end organs. Harrison (1910) showed that they could grow out freely *in vitro*. Hoadley (1925 *b*) found that when a portion of the brain and of the trunk region of a chick embryo are grafted together on to the chorio-allantoic membrane, nerve fibres develop and enter and innervate various structures. On the other hand, if only mesenchyme is present without other structures, the nerve fibres will not grow out far. The evidence is therefore all against the theory of pre-established connection between nerve and end organ, and this is proved conclusively by the following experiment. Harrison (1907) removed the nerve tube from an *Amblystoma* embryo and thereby destroyed all possible connection between nerves and limb buds. He next transplanted these "aneurogenic" limbs into the normal region of another embryo, where they became innervated in the normal manner. The nerves must therefore have grown out freely. Lewis' (1907 *c*) results also show that nerves can grow out freely. Detwiler (1920, 1922) grafted arm buds of *Amblystoma* into regions slightly distant from normal, and found that they still become innervated by the proper nerves from the normal level of the spinal cord provided that they are not too far distant. There must therefore be some chemotactic attraction of the nerves to the limb. If they are too far distant, their supply is abnormal. Similarly when the region of the nerve tube associated with the brachial plexus is rotated 180° (Detwiler, 1923 *b*), the brachial

plexus is identical with the normal. Transplanted limbs may function perfectly even when grafted from *Amblystoma punctatum* to *A. tigrinum* (Harrison, 1924), and when the spinal cord of *punctatum* in the arm region is exchanged for that of *tigrinum* (Wieman, 1926).

The organs of the lateral line were among the first to be studied. Harrison made use of Born's (1897) method of grafting together two portions of embryos belonging to different species. By grafting the head end of the dark-coloured embryo of *Rana sylvatica* on to the lighter coloured trunk of *Rana palustris* he was able to see that the lateral line grew back from the dark portion over the lighter (Harrison, 1904). It grew back at the proper level with regard to the side even when an intermediate piece of tissue over which it had to travel was rotated 180° either in the vertical or in the antero-posterior axis. Also when the head was grafted at an angle of 90° to the tail, the lateral line grew down till it reached the proper level and then grew back along it. Its path is therefore in some way governed; yet not absolutely rigorously, for if a scar was in its track, the line grew round it. This experiment is of the greatest interest in connection with Axial Gradients, when dealing with which it will be mentioned again.

Stone (1922) removed the vagus placode from embryos of *Amblystoma* and found that the lateral line did not develop; similarly extirpation of the preauditory placode resulted in deficiencies in the supra-orbital line.

In other experiments on *Amblystoma*, Stone (1925) planted the postauditory (vagal) lateral line placode in the place of the preauditory (facial) placode. It formed a definite supra-orbital line along the correct pathway, and the associated ganglion and nerve simulated the normal. When the facial placode was planted in the place of the vagal, it formed a lateral line which grew back in the proper place (although the placode had been rotated through 180°) but did not exceed the length which it would have reached in the normal (supra-orbital) position.

9. THE EYE, THE LENS AND THE CORNEA.

In Spemann's (1912 *b*) experiments already mentioned, in which a rectangular mid-dorsal piece was rotated at the open neural fold stage of *Rana esculenta*, the anterior cut edge usually went through the rudiments of the eyes, so that part of them were left in place, and part found themselves at the hinder end of the rectangular piece as a result of the rotation. This is proved by the fact that while a pair of small eyes were found in front in the normal position, another pair were formed behind, in front of or behind the ear vesicles according to the length of the rotated piece. (See fig. 6.) This shows in the first place that the eye rudiments were determined since they differentiated independently in strange surroundings. Further, since the sum of the sizes of the right fore and left hind eyes equalled that of the left fore and right hind, this determination was quantitative. When such eyes were very small, they had arisen from only a small part of the eye rudiment, which might consist only of tapetum pigment cells. Other eyes had no tapetum, and the rods and cones of the retina projected freely into the cavity of the brain: others again had too much tapetum, and this was thicker than normal. This means that the

various constituent parts of the eye are also qualitatively and quantitatively determined ("chemo-differentiated," Huxley, 1924) in the open neural fold stage; and the cuts in the experiment separated these constituents and distributed them unequally in the various cases. This was also the conclusion to which Lewis (1908) came when he transplanted the (still-invisible) presumptive eye rudiment of *Rana palustris* in the open neural fold stage. There were variations in the ganglionic layer, pigment layer, etc., which can be explained by an invisible determination of the various constituents at the time of transplantation. At the same time it is worth noticing that however deficient the eye is (within limits) it undergoes the morphological differentiation and regulation which makes it resemble a cup. Morphological and histological differentiation are therefore sharply distinct processes. Filatow (1926) cultured *in vitro* a portion of the eye rudiment of *Rana esculenta*. Both this piece and the piece left in the embryo regulated to form little eye cups. Similarly two eye cups in contact tend to regulate to one (Anastasi, 1913).

That the eye rudiment is self-differentiating at this stage, there is then no doubt. The most striking demonstration of this is Spemann's (1925) experiment in which he grafted the presumptive eye rudiment of a neurula of *Bombinator* into the flank of another. It developed in the body wall beneath the pronephros into an optic cup with the concavity towards the coelom. Other grafts in *Amblystoma* were made by May and Detwiler (1926). Filatow (1926) cultured *in vitro* the eye rudiment of *Amblystoma*. If taken from the open neural fold stage he did not succeed in getting it to differentiate. On the other hand, if taken from the stage at which the neural folds are first closed, it differentiates properly with a lens, almost keeping pace with the normal eye. Werber (1915) subjected embryos of *Fundulus* to acetone, and, among other abnormalities, obtained "meroplastic" embryos, in which only certain regions developed at all. In some cases this was a fragment of the neural plate, which developed by self-differentiation into a solitary isolated eye.

Strangeways and Fell (1926 *b*) grew the eye of the 64-hour chick in tissue culture, at which stage it consists of an outer layer one cell thick and an inner layer of epithelial cells. *In vitro* it differentiated into pigment, rods and cones, inner and outer nuclear layers, inner and outer plexiform layers, ganglionic cells and nerve fibres. Hoadley (1924) obtained differentiation of the eye of a 33-hour chick on the chorio-allantoic membrane.

Mention must now be made of some experiments in which Stockard (1910) subjected embryos of *Fundulus* to various solutions, such as alcohol, chloroform, ether and magnesium chloride, and obtained various degrees of fusion of the two eyes, a condition known as cyclopia. The median and intervening portions of the head appear to be missing, and only the lateral parts are well formed, though displaced towards the middle line to meet those of the opposite side. McClendon (1912) emphasised the fact that these results are not due to any specific action of the individual chemical compounds. He obtained cyclopia by using KCl, NaCl, NaOH, and cane-sugar. In the frog *Rana pipiens* Bellamy (1919) obtained fusion of the eyes, nasal pits, and ventral suckers by subjecting the embryos to LiCl.

Cotronei (1921 *c*) also treated embryos of *Rana esculenta*, *Bufo vulgaris* and *Triton cristatus* with LiCl, and obtained cyclopia, often accompanied by other abnormalities as regards the nose and mouth. In some cases in *Rana* and *Bufo*, he obtained anophthalmia. These results are comparable with those which Child (1924) has obtained in *Planaria*.

This raises an interesting question. In development, are the eye rudiments first of all median, only moving to the side later? If so Stockard's results are due to prevention of this lateral movement. Or are they from the beginning paired? In which case Stockard's cyclopic fish are to be explained by a deficiency of median material. Stockard (1913) tried to test this on *Amblystoma* material at the open neural fold stage. When lateral regions of the rudiment were removed 80 per cent. of normal eyes were obtained, while removal of median regions showed 48 per cent. without eyes. He therefore concluded in favour of the first alternative. Against this must be set the fact that Lewis (1909) by making a median injury in the embryonic shield of *Fundulus* obtained cyclopic embryos. Here clearly the result is due to median deficiency, not prevention of lateral movement. Further, Fischel, (1921) as the result of considering a series of cyclopic malformations in *Salamandra maculosa*, observed that the optic malformation was proportional to the nasal. He concluded that this parallel monorhiny would be inexplicable if cyclopia were due to non-separation of median eye rudiments, but must be due to median deficiency between paired rudiments.

Meanwhile, it may be noticed that in Spemann's (1921) experiments, in which a piece of strange presumptive epidermis was transplanted to the anterior presumptive neural fold region, the eye rudiment developed *in situ*; there was no movement away from the middle line. This result is conclusive. In other cases, Spemann (1904) found that one head of the two forming part of a duplicitas anterior might have malformations leading to and including cyclopia; and that this occurred when the plane of constriction caused by the noose of the ligature was slightly oblique with regard to the original plane of bilateral symmetry. One half was therefore slightly deficient in anterior material, leading to cyclopia.

A difficulty arises here. In Stockard's experiments the exposure of the embryos to the solutions causing cyclopia was at a time long before the determination of the rudiments of the eye (as proved by other experiments). How then can a deficiency be brought about in a rudiment which has not been formed? It may be noticed that the deficient region comprises what is morphologically the most anterior point of the embryo; *i.e.* that region which on the theory of Axial Gradients has the highest rate of protoplasmic activity. This region presumably is the first to suffer from the solutions in question, with the result that its relative rate is no longer maintained. Since there is reason to believe that qualitative differentiation depends partly on quantitative rate of protoplasmic activity (see p. 189), it is easy to see that suppression or rather lowering of the relative rate in the region where it is normally highest will result in the non-formation of the structures which would normally arise there.

This explanation will also account for Spemann's cases just mentioned. The

deficient half-embryo (it must be remembered that the embryo is ***not completely*** constricted into two, but the term "half" is used for facilitating description) lacks the most anterior region: the highest point of the gradient. At the same time, where the two heads join on to the common trunk is a region of definite rate which is the same for both. The "potential difference" in rate along the gradient between this point and the front end of the head which possesses the region of highest rate is sufficient to give rise to the normal structures there. But in the case of the other head which lacks the region of highest rate, the "potential difference" is insufficient, and cyclopia results for the same reason as in Stockard's experiments. The suggestion that these abnormalities (in Vertebrates) could be explained by means of Axial Gradients is due to Werber (1915) and Newman (1917).

The invagination of the optic vesicle to form a cup must be a self-differentiation. Ekman (1914 *b*) observed a case in *Rana esculenta* and in *Bombinator* in which the eye had two inpushings. Similar cases were found in *Salamandra* by Fessler (1920). Cotronei (1921 *a*) has shown that these apparent duplicities (which he obtained experimentally in *Bufo vulgaris*) are the result of mechanical conditions of available space and cell division inside the optic cup. They are in fact an exaggeration of a condition through which, according to Rabl (1917), the eye passes in its normal development in all Vertebrates.

It is now time to turn to the developmental mechanics of the lens, and as a prelude it is only necessary to stress the fact that although the eye cup and lens become so perfectly adapted to one another later on, they arise from quite separate rudiments, the lens being epidermal. It will be convenient to deal with the different species operated upon in turn.

Rana fusca seu temporaria. Spemann (1901 *b*) destroyed the eye rudiment at the open neural fold stage and found that although the presumptive lens tissue had not been touched, no lens developed. Filatow (1926) grafted the eye under the skin in the trunk region and obtained differentiation of a lens and he also (1924) obtained a lens from strange ectoderm over the eye *in situ*. Jenkinson (1906) subjected developing embryos to solutions of NaCl, NaBr, or $NaNO_3$, whereby the eye cups remained deep beneath the surface, and no lenses formed. On the other hand von Ubisch (1925) found that after removal of the eye a small "lentoid" might be formed, also after partial inhibition of eye development with Na_2CO_3.

Hyla arborea. Ekman (1914 *a*) grafted epithelium from a distance over the eye in the proper place, and obtained a lens. On the other hand the presumptive lens epithelium transplanted to other regions gave no lens.

Chick. Danchakoff (1924) grew a piece of the anterior region of a blastoderm at the head-process stage as a chorio-allantoic graft, and found that an eye cup differentiated and that a lens could be formed from strange epidermis, and perhaps from chorionic ectoderm.

Rana sylvatica. Lewis (1904, 1907 *a*) removed the eye and grafted it under the skin of the trunk, and obtained a lens.

Pelobates. Wachs (1920) found that after removal of the lens, the eye produced another from the margin of its cup.

Bufo vulgaris. Cotronei (1921 *a*) observed that in cases treated with LiCl the eye cup did not touch the skin, and the lens was not formed.

Filatow (1924) grafted non-presumptive lens epidermis over the eye and obtained a lens, also when it was grafted over the eye of *Rana esculenta*.

Triton taeniatus. Spemann (1905) removed the presumptive lens epithelium, and the gap closed by approximation of the sides of the wound. A lens was formed, therefore, from strange epidermis. But if the epidermis and the eye cup were separated by connective tissue, the lens was formed from the edge of the eye cup and not from the epidermis. Wachs (1914) also obtained a lens from strange epidermis.

Rana palustris. Lewis (1904, 1907 *a*) removed the eye and got no lens. King (1905) however found that a lens might be formed under these circumstances, but it was small. Strange epidermis over the eye produced a lens, even if it came from *Rana sylvatica*; and the eye grafted under the skin of the trunk induced a lens.

Bombinator pachypus. Spemann (1912 *a*) showed that removal of the eye might not prevent a large lentoid or slightly deficient lens from developing. Strange epithelium from the head grafted over the eye produced a lens of more or less normal size, but no lens could be obtained from epithelium from the trunk.

Amblystoma punctatum. Le Cron (1906) removed the eye at early stages and got no lens; on the other hand at later stages the lens might develop but was subsequently resorbed. Harrison (1919) showed that if the presumptive lens epidermis was removed, a lens formed from the regenerated tissue closing the wound, which has been in the immediate vicinity. On the other hand more distant epidermis grafted over the eye gave no lens. Presumptive lens epithelium grafted elsewhere before the neural folds close gives no lens. If it is taken after the closure of the neural folds, a lens does form. This is remarkable since extirpation of the eye at this stage prevents lens formation.

Rana arvalis. Filatow (1925) found that a lens would not develop from strange epidermis.

Rana esculenta. Removal of the eye does not prevent the formation of the lens, although it may be smaller than normal (Spemann, 1907 *a*). No other epidermis region grafted over the eye will give a lens (Spemann, 1912 *a*). Yet trunk epithelium of *Bufo vulgaris* grafted over the eye does give a lens (Filatow, 1925), and the eye of *esculenta* grafted under the skin of the trunk of *Bombinator* gives a lens (Spemann, 1908). Transplantation of the presumptive lens tissue may give rise to a lens (Spemann, 1912 *b*), but in these experiments (rotation of a rectangular mid-dorsal piece) it is noteworthy that no lens formed at the proper place if the eye were too small or did not touch the skin. In other cases the size of the lens was adapted to that of the eye.

Salmo. Mencl (1903) observed cases without eyes and in which lenses were nevertheless present.

Fundulus. Stockard (1910 *b*) induced artificial cyclopia, and found that the lens did not always conform to the eye. On the other hand when the lens was median

and single it probably arose from strange tissue, which led Herbst (1901) to suspect the correlation in development between eye cup and lens.

To all these must be added the fact that in *Rana fusca* (Bell, 1906), *Rana esculenta* (*ibid.*) and *Rana sylvatica* (Lewis 1904) the lens may be formed from the edge of the iris, as in certain cases of regeneration. It may be convenient to set out these results in tabular form (see p. 168).

Stockard (1907) has shown that in the development of *Bdellostoma*, contact between the eye cup and the epidermis results in the formation of a lens. Later, however, this contact is lost and the lens degenerates.

From all this it would appear that in one set of species the lens was dependent for its differentiation on the eye (*Rana fusca, sylvatica*; *Hyla arborea*; *Bufo vulgaris*; *Triton taeniatus*; *Amblystoma*); in another set independent (*Rana esculenta, arvalis*; *Fundulus* and *Salmo*) and self-differentiating.

It is obvious, as Spemann (1907 *b*) pointed out, that the various species of one genus cannot have radically different methods of forming the lens. There must therefore be some relation between self- and dependent-differentiation. In the first place it must be observed that the eye is in all cases capable of inducing a lens, for while in *esculenta* it cannot do so from its own strange tissue, it can from that of *Bufo* and *Bombinator*. The non-formation of a lens in *Rana esculenta* from strange epidermis grafted over the eye is therefore due to specialisation of the epidermis. In this connection it has been proved in *Amblystoma* and *Bombinator* that distant tissue will not produce a lens, while tissue nearer to the presumptive lens-site will. Now with regard to the power of a lens to self-differentiate it is to be noted that *Rana palustris* and *Bombinator* occupy an intermediate position, in fact even *Rana fusca* can sometimes produce a lentoid. Further, the experiments on *Amblystoma* show that the power of self-differentiation increases with age.

It may be concluded therefore that in all the amphibia at least, the lens is at some time dependent in its determination, but that in some groups this chemo-differentiation may occur very early, in the open neural fold stage (*Rana esculenta*), in others very late (*Rana fusca*). In all cases, however, contact of the eye with the skin appears to have some effect. Hoadley (1926 *d*) has made the interesting observation that in certain experiments on the chick, the lens is induced not by the optic cup (which has not yet been formed) but by the optic vesicle. This means that the inducing factor is independent of the degree of *morphological* differentiation reached by the eye, and must be connected with its *histological* or *chemical* degree of differentiation.

Von Ubisch (1922) has suggested that these differences can be explained with reference to temperature, since *Rana fusca* develops slowly (in cold weather), *Rana esculenta* rapidly (at a warmer time of year). Experiments show however (von Ubisch, 1924, 1925) that the self-differentiation of the lens is impeded in *Rana esculenta* at low temperature, in *Bombinator* at high temperature, while that of *Rana fusca* is more or less independent of temperature.

The differentiation and clearing of the cornea and conjunctiva must be considered before leaving the eye.

	Eye absent, removed or too deep	Strange epidermis over the eye	Eye grafted under strange epidermis	Presumptive lens material transplanted	Lens formed from edge of eye
Rana fusca	No lens, Spemann. No lens, Jenkinson. Lentoid, von Ubisch	Lens formed, Filatow	Lens formed, Filatow	—	Bell
Hyla arborea	—	Lens formed, Ekman	—	No lens, Ekman	—
Chick	—	—	Lens formed, Danchakoff	—	—
Rana sylvatica	—	—	Lens formed, Lewis	—	Lewis
Bufo vulgaris	No lens, Cotronei	Lens formed, Filatow	—	—	—
Triton taeniatus	—	Lens formed, Spemann, Wachs	—	—	Spemann
Rana palustris	No lens, Lewis. Lentoid, King	Lens formed, Lewis; also from *R. sylvatica*	Lens formed, Lewis	—	—
Bombinator pachypus	Lentoid, Spemann	Lens from head, no lens from trunk, Spemann	—	—	—
Amblystoma	No lens, Le Cron	Lens from near, no lens from far, Harrison	—	No lens if early, lens formed if late, Harrison	—
Rana esculenta	Lens formed, Spemann. Sometimes no lens, Spemann	No lens, Spemann. Lens formed from *Bufo* skin, Filatow	Lens formed in *Bombinator*, Spemann	Lens formed, Spemann	Filatow, Bell
Rana arvalis	—	No lens, Filatow	—	—	—
Salmo	Lens formed, Mencl	—	—	—	—
Fundulus	Lens formed, Stockard	—	—	—	—
Pelobates	—	—	—	—	Wachs

Lewis (1905), working on *Amblystoma* and *Rana sylvatica*, showed that early removal of the eye resulted in no cornea being differentiated. Spemann (1901 *b*) came to the same conclusion with regard to *Rana fusca* after removing the presumptive eye rudiment at the neural plate stage. Dürken (1913) also observed absence of conjunctival clearing in *Rana fusca* after removing the optic vesicle from a later stage. If the eye and lens are removed after the lens has separated from the skin, a small corneal area develops. The size of the cornea is adapted to the size of the eye when the latter is experimentally reduced. The eye can form a cornea in the absence of a lens. If the eye is removed soon after the lens is formed and the lens left, a small cornea is formed. Further, if the presumptive corneal epidermis is removed, a cornea can develop from the regenerated tissue. This result was obtained by Groll (1924) in *Rana fusca* also, when distant skin was grafted. He confirmed Dürken's (1913) observations that extirpation of the eye did not hinder the development of eyelids. Furthermore, he found after having differentiated, the cornea became opaque again if the eye was removed. The cornea therefore seems to require the eye not only for differentiation but also for maintenance. However, Luther (1916) removed the eye of *Rana fusca* and observed that nevertheless the cornea developed. Cole (1922) obtained interesting results from strange skin grafted over the eye in *Rana clamitans* and *catesbeyana*. In 60 per cent. of cases in which tail skin was grafted, there ensued a perforation and absorption, thereby exposing the eye. This absorption also takes place if tail skin is grafted over an artificial "eye" in the shape of a glass bead, showing that it is the result of the mechanical pressure caused by the curvature. These results are not obtained with skin from the back.

Fischel (1917) grafted the lens in *Triton* under the skin of the back. The gland cells disappeared, the area cleared and became a two-layered epithelium strongly reminiscent of a cornea.

Dürken (1916) extirpated the eye of *Rana fusca* and grafted a limb bud into the orbit. The conjunctiva became clear, which without the limb or the eye it would not have done. Here again, pressure appears to be the determining factor.

It is regrettable that authors have not always been precise in their usage of the terms cornea and conjunctiva. Lewis' (1905) paper is entitled "On the cornea," yet Groll (1924) refers to it as on the conjunctiva, as does Dürken (1916). Lewis himself (1905, p. 431) refers to "cornea or rather corneal changes of the ectoderm." At all events, it seems that the results described above apply to both the cornea and the conjunctiva *sensu stricto*.

10. THE EAR.

In some of Spemann's (1902) experiments on *Triton* in the open neural fold stage, it was found after transverse division that both portions of the embryo which developed contained small otic vesicles. The division must therefore have passed through the rudiments of the otic vesicles. Levy (1906) removed portions of the anterior regions in *Triton* embryos and obtained reduced otic vesicles. These experiments show that at this stage the otic vesicle is already determined. Further,

it is incapable of regulation, for Spemann (1910) divided a neurula of *Rana esculenta* transversely and found that while both portions contained rudimentary vesicles they were incomplete, since only one on each side had a ductus endolymphaticus. The various parts of the vesicle are therefore also determined. This is further supported by Streeter's (1907) discovery that the ductus endolymphaticus might be histologically normal when the rest of the vesicle was not. Sternberg (1924) grafted the vesicle of *Rana fusca* into the ventral gill region and observed self-differentiation of the ganglia and sense organs. Lewis (1906) grafted the vesicle of *Rana palustris* into *Amblystoma* and obtained self-differentiation. Hoadley (1924) obtained the same in the chick when the rudiment was grafted on to the chorio-allantoic membrane. Streeter (1909) found that two vesicles grafted together in *Rana* did not regulate into one. Eisinger and Sternberg (1924) removed the vesicle of *Rana fusca* and found that it was not regenerated. There is therefore no doubt that the otic vesicle at and after the neurula stage is self-differentiating and is further not an "equipotential harmonic system" (Driesch, 1921) but a "mosaic."

For facilitating the description of experiments, Harrison (1921 *b*) and Milojewic (1924) have introduced a terminology which may conveniently be described here.

Homopleural and Heteropleural denote whether the graft is planted on the side of its own origin, or on the opposite side.

Dorso-dorsal and Dorso-ventral indicate whether the dorso-ventral axis of the graft has been reversed in the experiment.

Antero-anterior and Antero-posterior give similar information with regard to the antero-posterior axis of the graft.

Medio-medial and Medio-lateral refer to the orientation of the medio-lateral axis of the graft.

A graft is Orthotopic if it is planted into a region similar or identical to that whence it was taken, if the site of implantation is different the graft is Heterotopic. In the case of limbs, an anterior limb graft in the region of the anterior limb is Homonomic, similarly a posterior limb graft in the region of the posterior limb. An anterior limb graft on the site of a posterior limb, or *vice versa*, is Heteronomic.

Turning now to earlier stages in the differentiation of the ear, Tokura (1925) found that in *Rana nigromaculata*, at the stage when the otic rudiment is just a thickening, rotation of this rudiment (*i.e.* homopleural dorso-ventral graft) leads to development in the reversed position, and also that a left rudiment on the right side (heteropleural) maintains its laterality. He further observed that if, at the stage before the otic thickening appears, the presumptive otic region is removed, a normal though smaller vesicle is formed from the neighbouring epidermis which has closed over the wound. This occurred in 17 out of 25 cases while the neural folds were still open. On the other hand, after the neural folds had closed an abnormally small vesicle was found, and only in 3 out of 25 cases.

In *Amblystoma*, Kaan (1926) has shown that before the otic cup is invaginated, the ectoderm for a considerable region round the site is capable of forming a vesicle, or even two more or less normal vesicles. After the otic cup has been formed, this power is lost by the other regions of the skin.

In order to ascertain what were the conditions in relation to the determination of the ear, Lewis (1906) removed the presumptive otic epidermal region and planted it back again, thereby destroying any connections with the underlying material. He also removed the cranial ganglia, or even the side of the brain, but in all these cases normal vesicles arose (in *Rana palustris*). Tokura (1925) also removed the side of the brain in *Bufo japonicus*, with the same result. It has not been possible therefore, at these comparatively late stages, to find a structure on which the otic vesicle is dependent for its determination, but it must not be forgotten that in an earlier stage grafts of organisers have the power to induce the formation of otic vesicles which otherwise would never have existed. Further, there must be some form of determination of the otic vesicle in the blastula, as shown by Dürken (1925 *b*).

Some very interesting results have been obtained by rotating vesicles through the various axes, and transplanting those from one side to the other.

Streeter (1906) transplanted the vesicle of the left side into the normal position on the right side in the frog. The result was that the vesicle maintained its laterality, but otherwise it assumed the correct positions, *i.e.* median side towards the brain, and dorsal side upwards; only the lagena pointed forwards instead of backwards, and the anterior semicircular canal projected caudally. Tokura (1924) found the same for *Rana nigromaculata*, which is indeed what would be expected from a self-differentiating organ. But the curious thing is that the dorso-ventral and medio-lateral axes should be correct, even when the graft had been heteropleural dorso-ventral. This is especially curious in cases where vesicles are rotated and left to develop on their proper side. Streeter (1914) found that if a vesicle were turned upside down and median side out it nevertheless tended to right itself (*Rana pipiens*). Ogawa (1921) found the same for *Rana palustris*, and for *Bufo japonicus* (Ogawa, 1922). Spemann (1910), however, rotated vesicles of *Rana esculenta* 180° about the transverse axis, and in most cases they developed in the rotated position with the ductus endolymphaticus pointing down instead of up. In one case the vesicle slipped back into the correct position. In *Rana nigromaculata*, Ogawa (1921) found that in 5 cases out of 9 the transversely rotated vesicle righted itself. Further (1926) he found that a half vesicle would right itself. This power decreases with age, and he was able to show that it is the age of the vesicle and not that of the surrounding tissues which limits the power, for rotation takes place when a young vesicle is grafted into an older larva.

This position regulation cannot be explained by the assumption that the ear is a harmonic equipotential system, for the many demonstrations of mosaic self-differentiation prove that it is not. It must therefore be due to a reverse rotation of the whole organ. This is not due to the relations of nerves to the vesicle, since Ogawa (1921) has shown that the righting takes place even when the rotation was performed at an early stage, before any nerves grew out. The suggestion that the ear rights itself because it only fits properly one way round in the neighbouring structures is disproved by the fact that a right vesicle adopts the "correct" position on the left side, although thereby there is a misfit between the lagena and the space

which normally accommodates it, and also because Ogawa (1921) has shown that *Rana* vesicles grafted into *Amblystoma*, and *Amblystoma* vesicles into *Rana*, can right themselves. In all these cases the rotation is gradual, and the cases of non-rotation and of retention of laterality show that the axes are fully determined in the rudiment. It is possible therefore that the vesicle rotates into the correct position either in relation to gradients in the organism, or in relation to gravitational stimuli. In this connection it is interesting to notice that the cases in which rotation of the vesicle is performed, and no righting occurs, lead to tadpoles which swim upside down and in all sorts of abnormal ways (Spemann, 1906 *a*). It is of interest to compare these processes of rotation in the ear with those which take place in the limb and girdle (see section 13). By a process comparable to that which gives rise to cyclopia, fusion of the auditory vesicles can be obtained under experimental conditions which lower the rate of protoplasmic activity at the anterior end of the organism (Reagan, MacMorland and Mudd, 1917). The power of the otic vesicle to induce the cartilaginous capsule is dealt with in the section on the skull, its power to induce a limb, in the section on limbs.

11. THE NOSE.

Ekman (1923) has shown that in *Rana fusca* the rudiment of the nose can be formed from tissue which has grown over to cover the wound caused by removal of the presumptive nose tissue; but more distant skin will not do so. Bell (1906) extirpated the nose rudiment in *Rana esculenta* and obtained the same result. In one experiment (Bell, 1907 *a*) he grafted the presumptive nose rudiment over the eye, in a 3·5 mm. embryo, where it self-differentiated. Lewis (1907 *c*) grafted the nose rudiment of *Amblystoma* just dorsal to the eye in another embryo. Differentiation continued and nerve fibres were given off. At the same time, in the correct position, a small nose rudiment was formed from the tissue which had closed over the wound. Hoadley (1924) obtained self-differentiation of the nose rudiment grafted on to the chorio-allantoic membrane. May and Detwiler (1926) showed that transplanted nose rudiments could self-differentiate in *Amblystoma*, while Spemann (1912 *b*) got the same result in *Rana esculenta*. In *Amblystoma* also, at the 5-mm. stage, Burr (1916 *a*) showed that after extirpation of the nose rudiment the neighbouring tissue could not form it. He also showed (Burr, 1916 *b*) that the nose is necessary for the regeneration of the fore brain. In Ekman's (1923) experiments on *Rana fusca*, it was found that however small and deficient the nose rudiment might be, if it reached the mouth epithelium at all, the latter responded by differentiating typical choanae. The latter are therefore dependent on the nose for their differentiation.

The relations of the nose to the olfactory cartilaginous capsule and to the mouth will be dealt with in the sections 14 and 16. The effects on cellular proliferation in the brain of grafting an extra olfactory organ on the head (Burr, 1924; May and Detwiler, 1926) have been dealt with in section 8.

12. THE PITUITARY.

Smith (1920) extirpated the hypophysis with knives from embryos of *Rana boylei* and *Bufo boreas*, 3½ to 4 mm. long, in the tail-bud stage. When the removal was complete, great changes were found in the infundibular constituents of the pituitary. Instead of showing the characteristic neuroglial thickenings, the infundibular floor was thin and membranous, the pars nervosa was diminished in size by 40 to 80 per cent., and was oval instead of being dumbbell-shaped.

When the removal of the hypophysis was incomplete, the remaining diminished portion of it might establish contact with the infundibulum in front of its normal position. It is noteworthy that in these cases the adjacent regions of the infundibular floor became thickened. The hypophysial portion did not however differentiate into pars anterior and pars intermedia unless it reached its normal position.

The neural elements of the pituitary are therefore dependent on the hypophysial for their proper differentiation, and the hypophysial are dependent on proper contact with the neural for theirs. It must be remembered, however, that in Mammals (Holt, 1921) a case is known in which the neural elements were properly differentiated in the absence of the hypophysial.

13. THE LIMBS AND GIRDLES.

To Braus (1905) belongs the credit of testing the powers of differentiation of the limb bud by grafting and transplanting.

Detwiler (1918) found that, in *Amblystoma*, the fore-limb rudiments are already determined at the neurula stage, and when transplanted they continue their development by self-differentiation. Harrison (1918) showed that the rudiment zone was in the form of a circle extending from the third somite to the middle of the sixth, and situated just beneath the pronephros. A normal limb can be formed from half a rudiment and from two rudiments fused together, so that it is a harmonic equipotential system. The cells round the rudiment have the power to form a limb, but with diminishing intensity as distance increases. Swett (1923), by means of an ingenious and laborious *intra vitam* staining method, was able to show that all the cells capable of limb formation did not take part in the production of a limb in normal development. By grafting the mesodermal tissues of the presumptive rudiment apart from their ectodermal covering, Harrison (1918) proved that the mesoderm is responsible for determining a limb, and Detwiler (1922) obtained normal limbs after grafting strange ectoderm over the presumptive limb region. The cells in the dorso-anterior quadrant of the rudiment have the greatest potency for limb formation (Harrison, 1918).

Great interest attaches to certain other experiments of Detwiler (1920) in which the limb bud was transplanted to other regions on the side at varying distances from the normal position. The cells round the original position gave rise to a small limb, but the degree of development which it achieved varied directly with the distance which separated it from the transplanted limb. The latter larger limb inhibits

the other if it is too near to it; *i.e.* within a certain range in which it is dominant. (See Child (1924) for other examples of physiological dominance.)

Attention must now be paid to certain experiments of Harrison (1921 *b*, 1925 *b*) which throw light on the process of determination. The limb rudiment can be regarded as a disc with anterior and posterior, dorsal and ventral and median and lateral, invisible axes. If a left limb bud is planted on the right side the proper way up and out (heteropleural dorso-dorsal antero-posterior), only the antero-posterior axis is reversed; as a result, it develops as a left limb. If on the other hand a left limb bud is planted on the right side the proper way out but upside down (hetero-

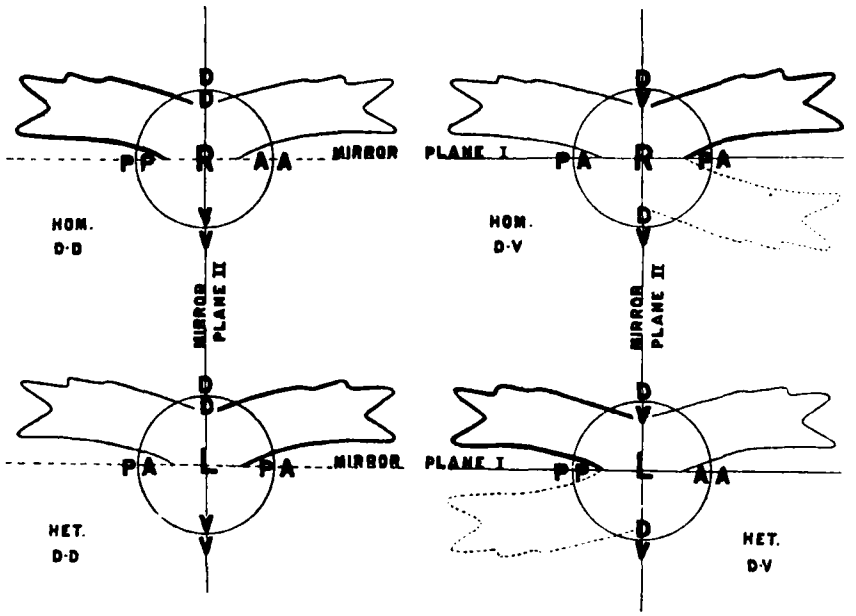

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Fig. 7. Diagram illustrating experiments on the symmetry of limbs. The circles represent the limb buds as grafted on to the *right* side of the body. The letters R and L in the centre of the circles indicate the side of origin of the bud (right or left). The letters A, P, D, V, *inside* the circle indicate the antero-posterior and dorso-ventral axes of the grafted bud; these letters *outside* the circle refer to the same axes of the body of the organism. The limb which develops is shown with a thick outline. The position of a reduplicated limb (should one develop) is indicated by the fine outline; the dotted line refers to the form which the limb would have taken if the dorso-ventral axis of the bud had been fixed at the time of grafting. Only medio-medial combinations are shown. (From Harrison.)

pleural antero-anterior dorso-ventral), only the dorso-ventral axis is reversed. Such a bud develops into a right limb the proper way up. This means that the antero-posterior axis is not reversible; that it has been already fixed and that it determines the anterior and posterior margins of the limb. On the other hand the dorso-ventral axis is not fixed, and is reversed and regulated to conform to the same axis of the body of the animal into which it is grafted. Similarly with the medio-lateral axis, for a left mesoderm-rudiment transferred to the right side with proper antero-posterior and dorso-ventral orientation (heteropleural dorso-dorsal medio-

lateral) but median side out, develops into a right limb. By rotating a right bud 180° about the medio-lateral axis and replanting it *in situ* (homopleural antero-posterior dorso-ventral medio-medial), the remarkable result is achieved that a right bud produces a left limb on the right side of the body. These experiments were performed on *Amblystoma punctatum* at the tail-bud stage.

Brandt (1924) has obtained the same results in *Triton taeniatus* (in which the limbs appear much sooner) only at the neurula stage; at the tail-bud stage in *Triton* all the axes are fixed. Ruud (1926) has further corroborated these results on *Amblystoma tigrinum*. Here the limbs appear relatively later, and yet the antero-posterior axis is determined earlier. There is therefore no correlation between time of determination and time of appearance of the limb.

It is not the transposition of material from front to back which is responsible for these modifications, for if a rectangular graft is taken and halved vertically, and the two halves interchanged without reversing their orientation, a normal limb results (Harrison, 1925 *b*). The effects are therefore due solely to the orientation of the axes.

In all these cases it was very common for additional duplicated limbs to arise from the transplanted buds, and the duplicates are mirror-images of the main limbs,

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Fig. 8. Axolotl in which an extra limb TR has developed from a grafted bud after homopleural dorso-ventral implantation. It is a "left" limb grown from a right bud on the right side. (From Harrison.)

and there may be two or more such duplicates on one limb; they may also arise at all levels on the limb. They obey Bateson's (1894) symmetry rule in that the long axes of the main limb and of its duplicate lie in the same plane, and each duplicate is with regard to the main limb, its image in a plane mirror bisecting the angle formed by the main limb and the duplicate at their point of junction, and placed at right angles to a line joining the corresponding structures of the main limb and the duplicate. The orientation and composition of these duplicates introduces the whole problem of asymmetry. Harrison (1921 *b*) is inclined to attribute asymmetry to an asymmetrical microstructure, perhaps of the molecular order.

Swett (1926) has further investigated the production of limb reduplications in *Amblystoma*. He finds that double limbs mirrored in the radial plane are much more numerous than those mirrored in the ulnar plane. Further, when there are two reduplicates, the radial one always develops first; when a reduplicate appears late it is always on the ulnar side. It is interesting to note in this connection that the

formation of digits takes place in a radio-ulnar direction, so that the radial border appears to be the more active of the two. In experiments in which a limb bud is

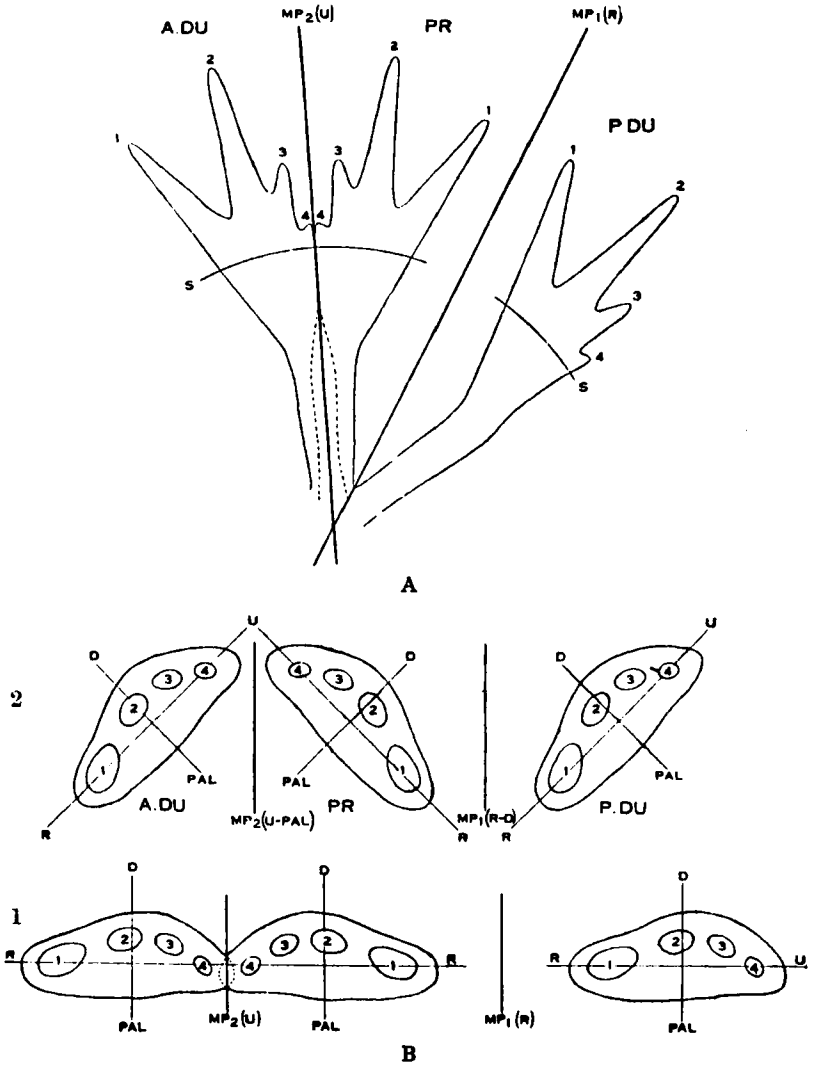

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Fig. 9. Diagrams illustrating the symmetry relations of reduplicated limbs, PR, primary limb; P.DU, posterior reduplicated limb; A.DU, anterior reduplicated limb; MP_1, radial mirror plane; MP_2, ulnar mirror plane; the figures 1, 2, 3, 4 refer to the digits.
A. Lateral view of the limb complex. B. Sectional view of the limb complex taken through S-S in A. In 1, the mirror planes are radial and ulnar (at right angles to the radio-ulnar plane). In 2, the mirror planes are radio-dorsal and ulno-palmar (diagonal to the radio-ulnar plane). (From Harrison.)

split into two by grafting in a strip of indifferent tissue, each portion may form a normal limb. These pairs of limbs will be situated anteriorly and posteriorly or

dorsally and ventrally according to the direction of the split which divides the limb bud. The remarkable thing is that the less normally situated of such a pair of limbs (each of which is of the correct asymmetry for its side) always forms a mirror-image reduplication on the side nearest the other limb. The latter in some way influences the former.

Nicholas (1924 *a*) grafted limb buds of *Amblystoma* into the mid-dorsal and mid-ventral line. The result was that two limbs developed from the bud, and if the antero-posterior axis was correct with regard to the body of the animal, these limbs had the proper asymmetry for the side on which they were; if the antero-posterior axis was reversed, there was a right limb on the left side, and a left one on the right. It may be noticed that these cases are similar to reduplications as regards their symmetry relations.

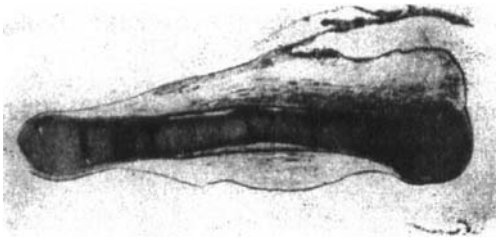

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Fig. 10. Longitudinal section of a femur developed from the proximal portion of a hindlimb bud of a chick incubated for 4 days. The bud was grafted on to the chorio-allantoic membrane of a 7-day chick and grown there for 5 days. Note the self-differentiation. (From Murray and Huxley.)

The important thing to note is that it is not laterality which is determined at this stage, but only the antero-posterior axis, *i.e.* the limb is determined in two but not in all three dimensions. The third dimension which makes the limb either right or left is determined by the dorso-ventral axis of the animal, regardless of the fact that by so doing the animal may develop a limb with the wrong asymmetry for the side.

The self-differentiation of limb buds is further well shown by Braus' (1905) experiments on *Bombinator*, in which he grafted the fore-limb rudiment (when it was still under the skin) on to the head, and into the place of an excised hind-limb rudiment. Wherever it was it developed into an arm, recognisable by the 4-fingered hand. Peebles (1910) extirpated the limb rudiment of the 4-day chick and found that no limb arose from any other cells. Strangeways and Fell (1926 *a*) took limb buds from the 81-hour chick, and grafted them under the skin of 11-day chicks. They differentiated into cartilage, bone, epidermis and fibrous tissue, but showed little relation as regards morphology to normal structure. They also cultivated the limb buds *in vitro*, and when grown in a rather solid medium they differentiated into cartilage, fibrous tissue and epidermis, bearing strong resemblances to a normal structure. No muscles were developed in either graft or culture.

Peebles (1910), using the 4-day chick, grafted the tip of the arm bud on to the

stump of the leg, and the tip of the leg on to the stump of the arm. It appeared that the grafts developed in conformity with their new situation, but the results are not clear. This means that regulation is still possible at this stage, and this result has been confirmed for regeneration-buds by Milojewic (1924).

The most definite results with regard to the determination of parts of the limb have been obtained by the method of grafting on to the chorio-allantoic membrane. Murray and Huxley (1925 *b*) took the basal part of the left posterior limb bud of the 4-day chick and cultivated it for 5 days as a graft. It differentiated into a perfect little left femur, with head and trochanter of cartilage with perichondrial bone. This experiment shows that a part of the limb bud is no longer capable of forming a whole at this stage. Its various regions are determined and are only capable of a very limited amount of regulation. This question has been further investigated by Murray (1926). The basal half of a 3-day limb bud differentiated into the proximal portion of a femur, while the apical half produced the distal portions of the femur, patella, tibia, fibula and foot. The following examples may be given of his experiments on the 4-day limb bud:

Basal half: complete femur; apical half: incomplete tibia, fibula, foot.

Basal half: proximal portion of humerus; apical half: distal portion of humerus, ulna, radius and hand.

Basal half: femur, proximal portion of tibia and fibula; apical half: complete (?) tibia, fibula and foot.

(If the tibia and fibula really are complete in the last case, then a certain amount of regulation must have taken place.) Basal quarter, perfect femur; second quarter, perfect tibia and fibula; apical quarter, perfect foot. Anterior and posterior halves likewise showed mosaic development. What structure differentiates in any given graft therefore depends on where the cuts were made in the limb bud: these parts must then be determined.

It is obvious therefore that after having been a partial equipotential harmonic system, the various regions become determined and the limb goes on to develop as a mosaic.

In several of these grafts, portions of the limb girdles appeared. Spurling (1923) found that in the 65-hour chick the separate parts of the pelvic girdle are already determined, and that removal of a portion results in a deficiency without regeneration. He also observed that the girdle rudiment at this stage would not regenerate a limb.

Braus (1909) transplanted the limb bud of *Bombinator*. In the place whence it came there developed only the distal ends of the suprascapula, coracoid and epicoracoid, and they were of normal size. The graft developed into a normal limb, and a perfect miniature shoulder girdle, one-third normal size. These transplanted portions of the girdle rudiment had therefore regulated. It is remarkable that the articulation between arm and girdle could not take place owing to size discrepancy, and yet each was perfectly formed. This is similar to Murray and Huxley's (1925 *b*) result with the chick femur, which had a well-formed head and trochanter. On the other hand, Meyer (1926) grafted the right arm bud of *Triton taeniatus*, at the

tail-bud stage, on to the left side of the head. The limb grew out normally, a shoulder girdle, deficient only in the suprascapula, was formed of one-third normal size, except for the glenoid cavity, which was of normal size, and fitted the head of the normal-size humerus. In this case, then, size regulation of the joint had taken place.

Harrison (1918) showed that in *Amblystoma* after extirpation of the limb-bud disc (3½ somites in diameter) portions of the suprascapula, coracoid and epicoracoid are left. These undergo hyperplasia and extend across the gap separating them. Detwiler (1918) showed that the separate parts of the girdle are already determined at the stage when the limb bud is a thickening of the body wall, and that the removal of a part was not followed by its restitution. The centre of the girdle rudiment is transplanted with an ordinary limb graft, and it develops into a girdle of one-third normal size. The dorsal half of the limb-bud zone is nearly free from the girdle rudiment. By transplanting portions of the bud, it is possible to obtain perfect limbs with deficient girdles.

At this stage, therefore, the girdle is not equipotential, while the limb is.

These results have been confirmed for *Triton taeniatus* by Brandt (1926).

Nicholas (1924 *b*), as result of experiments involving rotation of the limb bud, found that the symmetry of the girdle conformed to that of the limb, *i.e.* it might be wrong for its side, but it was not upside down. Harrison (1921 *b*) noticed that in some cases when the limb buds are rotated 180° and replanted on their own side, instead of developing with a reversed asymmetry, they underwent rotation at the shoulder joint, and eventually conformed to their side. Nicholas (1924 *b*) found that such limbs which had been rotated up to 235° righted themselves by reversed rotation. On the other hand, if they had been rotated through three right angles, they complete the circle by rotating the remaining right angle in the same direction. If the limb bud was only 1½ somites in diameter, no girdle is formed, and this regulatory rotation does not take place. If the graft was 5 somites in diameter, a complete girdle was formed, and no regulatory rotation takes place (Nicholas, 1926). When in a graft 5 somites in diameter the 3½-somite limb bud is separated from the rest, and both pieces are rotated independently, the limb undergoes postural regulation in regard to the peripheral piece, irrespective of its orientation to the organism as a whole (Nicholas, 1925).

Apparently the portions of the girdle (parts of suprascapula, coracoid and epicoracoid) whose rudiments are outside the 3½-somite disc, but are included in a 5-somite transplant, act as determining factors with regard to their orientation on those portions of the girdle whose rudiments are included in the 3½-somite disc. The girdle then determines the rotation of the limb. Obviously, then, in the 1½-somite graft, no girdle being formed, no rotation can occur; in the 5-somite graft, the whole girdle is rotated and remains so; in the 3½-somite graft, the central (transplanted) portions of the girdle unite with the normally orientated outer portions which had been left in the host, to form a complete girdle. This rotation of the girdle cannot take place as a whole, as shown by the fact that portions of pronephros transplanted with a graft do not move. So far then this rotation is a mystery.

That limbs can develop without nerve supply follows from Harrison's (1907) experiments already described in section 8 ("Aneurogenic" limb buds grafted after removal of the spinal cord of their embryo). Lebedinsky (1924) grafted a limb bud of *Pelobates* on to the ventral suface of the trunk in such a way that it hung by a long stalk. It developed normally, yet was demonstrably nerveless. Dauwart (1924) examined a reduplicated limb in *Pelobates* and found that it was also devoid of nerves.

Dürken (1916), on the other hand, from his experiments on *Rana fusca*, came to the conclusion that innervation was important for the development of grafted limb buds. The bud, consisting at the time of operation of mesenchyme and epidermis, was grafted into the place of the extirpated eye. It developed into either uninterpretable masses of cartilage and connective tissue, or into a more or less typical limb with musculature if it was innervated (by a branch of the trigeminal!). Other experiments by Groll (1924) appear to confirm this.

In other experiments, Dürken (1917, 1925 *a*) extirpated the leg rudiments of *Rana fusca* at a very early stage, and found that all three other limbs were deficient, as well as the nervous centres. Extirpation at a later stage did not produce this result on the limbs. The defects of the limbs were syndactyly, feebly developed toes, or long segments too long. Luther (1916) did not confirm these results, but they are partly supported by those of Hamburger (1925). He extirpated the right eye or the right optic lobe of the mid-brain in embryos of *Rana fusca*, and in 14 per cent. of cases the distal ends of the hind legs were deficient *in both legs*. The malformation took the form of suppression and reduction of digits 1, 2 and 5, the latest formed toes being the most affected. There can be no doubt that this effect is exerted through the nervous system (though non-specific, and probably due to general debility), and that innervation may therefore play a part in the later differentiation of the limb. It is interesting to compare these cases with those of hypotypic regeneration.

Mention must be made of three very curious sets of results which have been obtained in connection with limbs. Weber (1925) cauterised the hind-limb rudiments of one side in young tadpoles of *Rana fusca* with a fine platinum wire heated to redness. The results differed with the severity of the burn. If it was slight, the limb produced from the cauterised rudiment was one-quarter longer than normal. More severe burning resulted in a normal (or slightly smaller) limb, but the corresponding limb on the other side was a quarter longer than normal. When the burn was very severe, a very small limb or stump was formed and the corresponding other limb showed multiple reduplications. These experiments deserve repetition. Harrison (1924) made heteroplastic grafts between *Amblystoma tigrinum* and *A. punctatum*. The former species is the larger of the two, but limb buds appear first in the latter (in the embryonic period) and are well developed by the time when those of the former have made their first appearance. *A. punctatum* limb bud on *A. tigrinum* host gives rise to a limb which is not very remarkable, but a *tigrinum* limb bud on *A. punctatum* produces a limb which is at least twice the absolute size of the largest limb normally produced in either species.

The other puzzle arises out of Balinsky's (1925, 1926) grafting of an otic vesicle into the side of the trunk of *Triton cristatus*, at the late tail-bud stage. In certain cases a limb developed from the site of implantation. The possibility of a limb bud having been grafted with the ear is excluded. It is worth noticing that such a limb was nerveless. It is known that the otic vesicle has the power of inducing the formation of cartilage round itself, and perhaps the quality of such cartilage is determined by the region or "field" (Weiss, 1925) into which the otic vesicle is grafted.

Leaving the pentadactyl limb and turning to the fins of fish, some interesting and important results have been obtained by Braus (1906 *a*) in experiments on the Selachians *Scyllium* and *Pristiurus*. The first appearance of the fin is in the form of a fold of skin into which the muscle buds wander. Later on the cartilaginous radials appear, parallel to the muscles. Braus' first object was to see whether the formation of the cartilages was dependent on the muscles. To this end he cut a slit along the base of the fin parallel to the side of the body, and thereby prevented the muscle buds from entering. Nevertheless the cartilaginous radials developed. The latter normally appear first at the centre of the fin, and develop in succession, forward and backward. In order to find out whether this was a causal sequence or merely a temporal one, Braus made a cut in the fin perpendicular to the axis of the body. This did not prevent the entry of the muscle buds, and the formation of radials proceeded regularly up to the slit but no further. Beyond it there was merely an undivided plate of cartilage instead of separate radials. There is therefore good evidence that an impulse for the differentiations of cartilaginous radials travels, in some form or other, over the fin.

14. THE SKULL.

The results of experimental work relating to the skull are not numerous, though of considerable interest. Stone (1922) removed the neural crest from *Amblystoma* embryos at the tail-bud stage, and obtained deficiencies in the visceral skeleton corresponding to the region which had been removed. More recently (Stone, 1926) he has shown that removal of the neural crest and "mesectoderm" in the region of the trigeminal nerve results in great deficiencies in the palato-quadrate, Meckel's cartilage and the trabecula in front of the optic foramen, on the side operated upon. These remarkable results are difficult to interpret, as it is possible that the tissues removed may not themselves be the future constituents of the cartilages in question, but may influence their formation in some way. At the same time, by transplanting portions of the neural crest to the trunk, Stone obtained differentiation of cartilage. However, it is very interesting to see in these experiments support for the old suspicion that the trabeculae may be of visceral origin, and similar in nature to the jaws and arches.

At later stages these regions of the skull appear to be self-differentiating. Schaper (1898) removed the dorsal portions of the brain with the eyes and ears of newly hatched tadpoles of *Rana esculenta*. The wound healed and in the course

of a week the tadpoles had grown 2 mm. and it was found that the pterygo-palatine and the gill arches had differentiated properly.

Steinitz (1906), after removing the eye of a frog embryo, observed that the skull was malformed, but that the optic foramen was present though no optic nerve pierced it. It was however smaller than normal.

Burr (1916 *a*) removed the rudiment of the nose from embryos of *Amblystoma* 5 to 6 mm. long. Normally the nasal capsule is a cast of the nasal sac, but in these cases the capsule was completely collapsed. The cartilages were there, having self-differentiated from the mesenchyme, but they are dependent for their conformation on the nasal sac.

The cartilage of the auditory capsule has been proved to be dependent on the presence of the otic sac. Filatow (1916) found that removal of the otic sac from *Bufo* resulted in absence of the cartilaginous capsule. Reagan (1917) extirpated the otic sac from chick embryos; the capsule and the stapedial plate did not develop, the columella auris did. When displaced into strange mesenchyme, the otic sac induced the formation of a capsule. This result has been confirmed by Luther (1925) in *Rana esculenta*. The auditory capsule, operculum and pars interna plectri were shown to be dependent on the otic sac, the annulus tympanicus, pars media and pars externa plectri independent. Eisinger and Sternberg (1924) showed that even a small remnant of the vesicle is sufficient to induce the formation of cartilage.

Luther (1925) transplanted the otic sac to a region between the eye and the ear in *Rana esculenta* and obtained differentiation of cartilage: on the other hand if the graft was made into the trunk region, no capsule was formed. Filatow (1916) transplanted the otic sac from younger to older embryos of *Rana fusca*, and observed that the cartilage was formed from the tissues of the older host. Similarly Lewis (1906) grafted the otic sac of *Rana palustris* into *Amblystoma* and observed that the cartilage induced was formed from *Amblystoma* material. Stone (1926) however maintains that when an otic vesicle is transplanted in *Amblystoma*, a cartilaginous capsule only forms if mesectoderm is transplanted with it. This is improbable in view of Filatow's and Lewis' results.

Balinsky (1925, 1926) grafted the otic vesicle of *Triton cristatus* into the trunk at the late tail-bud stage, and in some cases obtained a limb. This result makes one wonder whether the cartilage which developed when Sternberg (1924) grafted the otic sac of *Rana fusca* to the ventral gill region might not be an attempt to form a visceral arch. It is perhaps worth mentioning that when Meyer (1926) grafted the limb bud of *Triton* on to the head, the auditory capsule was markedly smaller on the side of the graft. The relation of the palato-quadrates to the balancer in Urodeles is described in the next section.

15. THE GILLS, THE OPERCULUM AND THE BALANCER.

Harrison (1921 *a*) working on *Amblystoma* rotated the presumptive gill ectoderm at an early stage and obtained normal gills. At a later stage, however, rotation was followed by self-differentiation. The determining factors must lie in the deeper

tissues because transplantation of ectoderm from the head, heart and pronephric regions over the presumptive gill region gave rise to normal gills. On the other hand, trunk ectoderm could not be induced to form gills. On the whole the closer the seat of the origin of the graft to the normal gill region, the more perfectly does it develop into gills. At the later stage, when rotation through 180° leads to growth in the rotated position, the underlying tissue cannot be a well-defined mosaic, because two rudiments grafted together regulate to form normal gills provided that their antero-posterior orientation is correct.

These results are thoroughly supported by the experiments of Ekman (1913, 1922) on *Rana fusca*, *esculenta* and *Bombinator*. He found that if at the stage when the neural folds are just visible, the gill region was rotated 180°, normal gills were produced. If this experiment were repeated at a time between the neural plate and the gill plate stages, the gills developed reversed, and the operculum grew forwards instead of backwards. Other ectoderm in *Bombinator* grafted over the gill regions will give rise to gills provided that it comes from the head, heart or pronephric region, but not from the trunk. In *Rana fusca* and *esculenta*, however, any ectoderm can be made to form gills. After the first origin the circulation plays an important part in gill development, which is impeded on removal of the heart. Stöhr (1925) found that in *Bombinator*, stoppage of the afferent branchial circulation on one side resulted in under-development of the gills on that side.

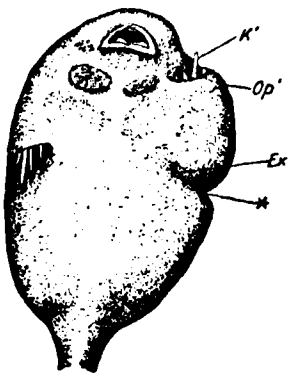

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Fig. 11. Larva of *Bombinator*, 3 days after the ectoderm of the gill region on the left side had been rotated through 180°. • limit between the rotated and the normal ectoderm; *Ex*, position of the forelimb; *Op'*, opercular fold; *K'*, gills. Note self-differentiation in the rotated position. (From Braus after Ekman.)

It may be remembered that Spemann (1921) showed that in *Triton* at the middle gastrula stage, presumptive nerve tube material of *taeniatus* grafted on to the gill region of *cristatus* proceeded to differentiate into gills, only it retained the *taeniatus* character of (precocious) development.

In Anurans when the arm develops it is covered over by the operculum, and in order to emerge the right arm perforates the operculum with its elbow. Braus (1906 *b*) showed that in *Bombinator* extirpation of the limbs was still followed by a perforation of the operculum which was smaller than normal. Ekman (1922) obtained the same result in *Rana arvalis*, Helff (1926) in *Rana pipiens* and *sylvatica*, and Braus (1920) in *Rana esculenta*, the perforation here being of normal size. These results were held (though as will be seen erroneously) to prove that the perforation was self-differentiating. At the same time Banchi (1905) had grafted an extra limb bud under the operculum in *Bufo*, and it induced its own perforation. The perforation was therefore dependent-differentiating also!—another alleged case of double assurance.

Later experiments have however shown that the perforation does not occur independently, though there is still confusion between the results.

Weber (1923, 1924) extirpated the arm bud in *Bombinator* and found that the perforation in the operculum formed if the limb stump were covered with glandular epithelium, but not otherwise. Helff (1924) paid attention to the fact that normal perforation of the operculum is accompanied by autolysis. Now when Braus (1906 *b*) grafted arm buds under the skin of the head, or Helff (1926) grafted them under the skin of the back, or even inserted glass beads under the skin, perforations occurred but without autolysis. At the same time all skin is capable of autolysis, as was proved by grafting back and side skin to the opercular region. (Helff's experiments were on *Rana clamitans*, *pipiens* and *sylvatica*.) Obviously then some structure other than limbs but near them is responsible for autolysis, as opercular skin grafted on to the back does not undergo autolysis. This structure has been proved to be the atrophying gills, for by transplanting resorbing gills beneath opercular skin on the back, autolysis was obtained. It appears then that the developing limbs accelerate the perforation (and localise it) by pressure. These cases show very well how provisional the description "self-differentiation" is.

The balancer is an organ present in some newts at the side of the head, and in the form of a long ectodermal tube with a mesodermal core. Bell (1907 *b*) found that in 3-mm. embryos of *Diemyctylus*, the ectoderm can differentiate into a balancer when transplanted without mesoderm. The determination therefore lies in the ectoderm, which induces the formation of a mesodermal core. It will only be regenerated if injuries are inflicted on its rudiment before it begins to grow out, *i.e.* early enough.

Harrison (1925 *a*) has confirmed these results in *Amblystoma punctatum*. At the neural fold stage its rudiment can be transplanted elsewhere on to the head, and it will develop by self-differentiation. *Amblystoma tigrinum* does not possess a balancer, but when a rudiment from *A. punctatum* is grafted on to it, it develops. Other epidermis grafted over the balancer region will not produce it. The degree of determination (for a long time invisible) becomes more and more pronounced with time; from young embryos the rudiment will only develop if grafted on to the head, from older ones the rudiment (still invisibly determined) will differentiate even if grafted on to the trunk. In grafts between embryos of different stages the balancer retains the "age" of its own original embryo.

The nerve supply is normally a twig of the mandibular branch of the trigeminal; and it can be innervated by this nerve in *Amblystoma tigrinum*, or by any other cranial or spinal nerve, and even by the trigeminal of *Rana sylvatica* when grafted on to this host.

At a late stage of development the balancer drops off, its basal membrane at its base becomes then embedded in the mesenchyme, and a cartilaginous extension of the palato-quadrate runs to meet it. Absence of the balancer entails absence of this process, as normally in *Amblystoma tigrinum*, in which however it may develop when a balancer is grafted on to it.

16. THE GUT, THE HEART, THE BLOOD, THE LIVER AND PANCREAS.

One of the results of Spemann's (1918) experiment on *Triton taeniatus* in which at the gastrula stage a piece of presumptive nerve-tube tissue together with the underlying gut roof was rotated through 180° and replanted, was that the embryo, which was perfect in all other respects, showed situs inversus viscerum et cordis (*i.e.* the stomach is on the right, the liver on the left, and the heart is twisted in the direction opposite that in the normal). Pressler (1906) obtained similar results in *Bombinator*, and Meyer (1913) in *Bufo vulgaris* and *variabilis*. Since the ventral regions of the gut had not been touched by the operations, the fact that not only the gut but also the heart was inverted as regards their asymmetry, shows that the latter is governed by some factor at this stage situated in the gut roof.

As a result of the ligaturing of *Triton* embryos in the gastrula stage leading to duplicitas anterior, Spemann and Falkenberg (1919) found that in 10 out of 12 cases, the right member of a pair was inverted, the left one normal. When the ligature was performed at an early stage and carried right through so as to give separate embryos from each lateral half of the gastrula, Ruud and Spemann (1923) found that the embryos developed from the left halves were normal, but half the number of those developed from the right half-gastrulae showed situs inversus. Wilhelmi (1921) suggested that this was due to the absence of a factor situated on the left side and whose function it was to determine the normal asymmetry. The left halves naturally possess this factor and are normal; the right ones lack it, and in these chance decides which way their asymmetry shall lie. She also extirpated regions from the left side and obtained situs inversus. This factor would also be supposed to function in the cases of the rotated gut roof, which in Spemann's (1918) experiments was a median piece. But when Meyer (1913) removed this piece of gut roof altogether, the hypothetical factor was absent, yet no situs inversus occurred. In the absence of such a factor it would be expected that half such animals would show situs inversus. The essential difference between Meyer's and Wilhelmi's experiments was that in Meyer's the deficiency of tissue was equal on both sides, in Wilhelmi's the deficiency was restricted to the left side. Warynsky and Fol (1884) obtained situs inversus in chicks by overheating on the left side. The result is that the right side is favoured.

Spemann's analysis of the question shows that the asymmetry of the viscera and heart may be due to: (i) an intimate asymmetrical microstructure, or (ii) a deficiency of material on one side. Since normally situs inversus is rare, and since artificial determination of the plane of symmetry (see Born, 1885; Jenkinson, 1909) does not lead to situs inversus, the asymmetry of the viscera must be determined at the time when the plane of bilateral symmetry is determined. Further, it is inconceivable that an external factor at this stage should always influence the egg in the direction of the same asymmetry; it is necessary to conclude that this determination lies with an intimate asymmetrical microstructure. This may refer to the sperm or to the egg structure itself. Reversal of this structure may cause

situs inversus. But Ruud and Spemann's and Wilhelmi's experimentally produced cases of situs inversus *need not* be due to reversal of the asymmetry of the microstructure. The hypothesis that lack of material on one side may invert the viscera covers all the facts; in the left gastrula halves the lack is on the right which accentuates the normal asymmetry; in the right halves the lack is on the left, and this may reverse the normal asymmetry. Similarly in Wilhelmi's experiments, extirpation on the left side causes lack of material on that side. Further, the "lacking" side often has smaller eyes, limbs and body muscles. On the other hand, the results of rotating the gut roof seem to require the assumption of a localised factor for their explanation.

Mangold (1921) has attacked this problem very subtly. He found that normally about 2 per cent. of individuals of *Triton taeniatus* have situs inversus. It is known that 50 per cent. of right half-gastrulae have situs inversus. If this is due to reversal of the asymmetry of an intimate microstructure, then if the two blastomeres of the 2-cell stage be separated, 50 per cent. of the individuals developed from the right-hand blastomeres should be inverted. As a matter of fact only 3 per cent. are, a proportion very similar to the normal. These cases are therefore probably not due to reversal of an intimate microstructure. On the other hand, it is possible that the microstructure gets stronger in its effects as development proceeds, in which case there would be no reason to expect situs inversus from right halves at early stages. However, there is no reason why the microstructure factor should not coexist with the lack-of-material factor. This must indeed be so, for taking these cases of half-gastrulae and calling the microstructure factor A, the other B, then:

(i) That the asymmetry is normally constant can only be explained by A.

(ii) That only the right half-gastrulae show situs inversus can be explained by either A or B.

(iii) That not all the right half-gastrulae are inverse is only explicable by B.

Further support for the view that asymmetry and its reversal may be caused by either A or B is obtained from a consideration of Swett's (1921) observations on double trout monsters. Naturally only those monsters which are separate at least as far back as the hind end of the stomach can have two stomachs and show situs inversus at all. The important point is that those twins which are joined together only by the hinder region of the trunk (behind the abdominal cavity), or by the tail only, or which are quite separate, very rarely show situs inversus; whereas those which join together behind the stomach and in front of the end of the abdominal cavity frequently do in the right hand member. Some factor such as diminished material or diminished protoplasmic activity is therefore at work in the latter cases, where the asymmetrical regions in question are in close contact, to overcome the normal superior development of the left side, which must ultimately be due to an intimate microstructure. When these regions are quite separate, this disturbing factor is absent and the effects of the microstructure factor continue unmolested. In the beginning, probably all that factor A does is to confer a higher rate of activity and growth on the left side of the organism; and if as seems very probable this "dominance" of the left side increases during development, the time factor must be taken into consideration.

When the heart is inverted, the gut is so too in all cases, but the converse is not true. This shows that the asymmetry of the heart is dependent on that of the gut and suggests that it is not due to an asymmetrical microstructure, since it would be difficult to imagine it normal in one and reverse in the other.

Turning now to the determination of the heart, Stöhr (1925) proved that in *Bombinator* at the neural plate stage the heart rudiment could be rotated through 180° and still give a normal heart, while this is no longer possible at the tail-bud stage. Ekman (1921) showed that when the whole heart rudiment was removed at the neural plate stage, a heart was formed from neighbouring tissues, but not at a later stage. If at the neural fold stage a lateral half of the heart rudiment be removed, the remaining half gives rise to a normal heart, though in one case out of eight the right half gave an inverse heart (Ekman, 1925). Stöhr (1925) halved the rudiment at the neural plate stage, left one half in its embryo and grafted the other into another embryo from which the whole rudiment had been removed. The result was a normal heart in both embryos. Ekman (1925) grafted a piece of the lateral pharynx region into the heart rudiment in an early neurula, and observed (by means of *intra vitam* staining) that it took part in the formation of the heart. Power to be included in the heart decreases with distance from it. The heart rudiment can be augmented by planting in part of another; if this is done at the neural plate stage the whole will regulate to form a normal-sized heart, whereas at the tail-bud stage the heart so formed is abnormal.

After implantation of a piece of heart rudiment rotated through 180° into a slit in a heart rudiment at the neural plate stage, regulation does not take place and two hearts are formed, the right inverse. Ekman (1924) has obtained two and even three hearts in an organism by making longitudinal slits and preventing the cut edges from rejoining one another. At the neural plate stage, then, the heart rudiment is a harmonic equipotential system.

Stöhr (1924 *b*) showed the self-differentiating capacity of the rudiment by transplanting it to various positions in other individuals. The heart so formed enters into the circulation and pulsates independently of the host's own heart. It is remarkable that when grafted into abnormal positions the heart may grow to twice its normal size. The heart will also develop even if deprived of its blood supply, only it is then smaller than normal.

Results very similar to those just described in Anura were obtained in *Amblystoma punctatum* by Copenhaver (1926) who showed that any part as large as a half of the heart rudiment can form a complete heart, one rudiment can under experimental circumstances produce two hearts, and that two rudiments can regulate to form one heart, at the early tail-bud stage. It is therefore a harmonic equipotential system, but even at the earliest tail-bud stage the rudiment is already determined as regards the antero-posterior axis, as experiments of rotation show.

It will be remembered that in Murray and Huxley's (1925 *a*) grafted anterior third of the 24-hour chick blastoderm, the various regions of the heart must have been determined. In their specimen the bulbus and ventricle were present, but not the auricle or sinus.

Remarkable results have been obtained by culturing heart rudiments *in vitro*. Ekman (1921) found that explants from the neural plate stage of *Bombinator* and *Rana esculenta* differentiated into sinus, auricle, ventricle and bulbus, and pulsated about 35 times a minute. Stöhr (1924 *a*) observed however that such hearts do not have the proper torsion.

It is customary to regard the blood as an "organ," yet it is interesting to find that in development in the frog it has a specific, definite and localised rudiment. Frederici (1926) extirpated the median ventral blood island from embryos of *Rana fusca* at the early tail-bud stage. If the extirpation was complete, the embryo had no erythrocytes, and in cases of partial extirpation, the amount of erythrocytes present was proportional to the amount of the rudiment which was left.

Holtfreter (1925) found that the rudiments of the liver and pancreas were already determined at the late gastrula stage. When implanted in the yolk mass of another embryo in the tail-bud stage they develop by self-differentiation. The tubules of the liver develop if blood is present. It is remarkable that the rudiment of the gall bladder is not localised at this stage, for it develops from grafts of the pars hepatica of the liver as well as from those of the pars cystica.

The development of the rudiments of the viscera of the chick has been described in section 7.

Adams (1924) has observed that in *Amblystoma* the mouth does not open unless there is contact between the ectoderm and endoderm of the oral plate. It may be recalled that Ekman (1923) showed that in *Rana fusca* the stimulus for the formation of the choanae came from the nose. Cotronei (1921 *b*) obtained malformations of the mouth in *Rana* and *Bufo*, as a result of treating the embryos with LiCl.

17. DEPENDENT DIFFERENTIATION AND SELF-DIFFERENTIATION; AXIAL GRADIENTS.

One of the most interesting questions arising out of consideration of the evidence presented in the foregoing pages, refers to the relation between the dependent and independent methods of differentiation of an organ. This question has been specially treated by Spemann (1907 *b*), Becher (1912), Braus (1914), Brachet (1914) and Dürken (1919), to mention only a few.

In many organs (*e.g.* lens of *Amblystoma*, gills of *Amblystoma*, *Rana* and *Bombinator*, nose, heart) it is plain that at first they are dependent on something else for their determination, and that later they acquire a degree of independence which increases with age. This means that the processes of chemo-differentiation take time, and, according to the period when the organ is tested, it shows dependent or self-differentiation.

There is no need to have recourse to the inheritance of acquired characters, or rather transmission of self-induced modifications, in order to explain the case of the opercular perforation. It is unnecessary and erroneous to speak of the cells of the operculum having become so accustomed to perforate during the course of countless generations that they can do so in the absence of the limb, when it is known that perforation is no induced self-differentiating phenomenon, but is simply

dependent on the presence of the resorbing gills. Enough is known about the lens to suspect that it also at the start is dependent, even in *Rana esculenta* where its independence has already been achieved at the open neural fold stage. There is therefore no need to imagine either that the lens' self-differentiation is an induced and transmitted somatic modification, or that the separate determinations of eye and lens owe their interadaptiveness to a miraculous chance. On the contrary, it is becoming clearer all the time that correlations of this kind are due to chains of events occurring in *Ontogeny*, not in Phylogeny, and there is no basis for "double assurance" of the type discussed by Braus (1914) and Brachet (1914) among others.

In the final analysis nothing is self-differentiating, not even the organism itself. Child (1924), in a masterly sequence of reasoning, has shown that the origins of determinations, the most fundamental of which are polarity and symmetry, cannot have their sole explanation in internal factors. Since nuclear division is known not to be unequal, the complete idioplasm is present in every cell, and countless experiments have proved that the tissues of one region can have the potency to give rise to those of another. A special internal factor which would determine that such and such a region should become head and such another right side, falls under the same objection that it too would be distributed all over the organism.

As a matter of fact it is known that these early determinations are due to the environment; the apico-basal axis to the orientation of blood vessels in the ovary, the plane of bilateral symmetry to the entrance of the sperm. Only in the case of the normal visceral asymmetry does it appear to be necessary to appeal to an intimate microstructure, but if the latter resides in the sperm, then it too is external to the egg.

Given polarity and symmetry the only hypothesis which appears tenable is that of a Gradient System or "field" (Weiss, 1925) both for the origin of this polarity and symmetry, and for the place of determination of the rudiments of other organs. The most important feature of the theory is that qualitative differentiations are controlled by quantitative differences of rate of activity of protoplasm reacting on specific genes. This rate varies along a gradient, and at different "levels" on the gradient certain determinations take place. Before the frog's egg is fertilised, it is ready to form with its ventral side in *any direction*. This is completely unintelligible if the rudiments of the organism are internally self-differentiating. The very determining of the rudiments must bear a relation to the stimulus which fixes the plane of bilateral symmetry. As mentioned before, the first rudiment to be irrevocably determined is the organiser.

It is no part of the purpose of this review to enter in detail into the theory of Axial Gradients. It is, however, pertinent to enquire whether any of the evidence dealt with supports it. The following points are of interest in this connection:

(i) Huxley's demonstration (1926) that a temperature gradient passed through a frog's egg, translated into terms of protoplasmic activity, caused a modification of cell size in the blastula, of the rate of growth of the animal pole cells in the gastrula, and of the relative head size of the tadpole.

(ii) Bellamy's (1919) demonstration of regions of high protoplasmic activity

at the animal pole and dorsal lip of the blastopore in the frog's egg by various methods. These results have been criticised by Cannon (1923) but confirmed by Bellamy and Child (1924). (See also Hyman, 1921, 1926, 1927.)

(iii) Geinitz' (1925 *b*) observation that secondary embryos induced in *Triton* by organiser implantation are properly orientated in the antero-posterior axis with regard to that same axis of the host.

(iv) Spemann and Mangold's (1924) and Bautzmann's (1926) observation that in these organiser grafts, corresponding organs of the primary and secondary embryos tend to be at the same level with regard to the animal pole.

(v) Harrison's (1904) discovery that the path of the lateral line in frogs during its backward growth is not locally predetermined, and yet bears a definite position-relation to the whole organism, on an antero-posterior gradient, at a certain dorso-ventral level.

(vi) Harrison's, Ekman's, Stöhr's and Copenhaver's discoveries that in the rudiments of the limb buds, the gills and the heart, the first determination is that of the antero-posterior axis.

There is therefore strong support for the view that the various rudiments become determined at certain levels in a gradient co-ordinate system of which the abscissa culminates at the organiser and the ordinate at the animal pole. Gradient-determination is followed by progressive and soon irreversible chemo-differentiation *in situ*, as is proved by the increasing degree of independence of rudiments with age. In other words, this period in a rudiment represents the transition from the dependent to the independent differentiating condition.

In this manner it may be imagined that the rudiments are localised: gills, balancer, nose, ear, placodes, hypophysis, lens, limbs, liver, pancreas, heart and perhaps the nerve tube. At the same time it is quite clear that these segregates do not possess definite boundaries, but are to be regarded as centres of intensity of determination. This is shown very clearly by the fact that tissue capable of forming an organ does not form part of it in normal development (*e.g.* limb, lens).

Now that some of the activities and properties of the organiser are known, it may be asked whether the organiser functions as a self-sufficient, totipotent creator of differentiation, or whether it works on material which is already heterogeneous. The latter alternative is undoubtedly the correct answer to this question, but it must be understood that this heterogeneity is not of the nature of chemical differences of substance, but quantitative differences of rate of activity. The evidence for this may be briefly summarised as follows:

(i) Corresponding organs of primary and secondary embryos occur at the same parallel of latitude with regard to the original egg axis. This means that future potencies are already graded in respect of the primary apico-basal or antero-posterior axis. The same conclusion can be drawn from the fact that in normal development any given organ will always arise on a definite parallel of latitude whatever its meridian of longitude may be, the latter being determined by the point of entry of the sperm, or what comes to the same thing, the organiser.

(ii) Secondary embryos induced by an organiser implantation tend to lie along a meridian of longitude in respect of the original egg axis. This means that the

secondary medio-lateral gradients are at right angles to the egg axis, and determine the relations to the middle line of the organs which arise at any given parallel of latitude.

(iii) Raising or lowering the relative rate of protoplasmic activity at the top of the gradient results in an alteration of the proportional sizes of the head and the body. Thus in Huxley's (1927) experiments on the frog the head size was affected by heating or cooling the animal pole. Gowanloch's experiments (quoted by Child, 1924) on the fish *Macropodus viridi-auratus*, make use of the differential susceptibility of various regions to sub-lethal concentrations of toxic solutions (in this case atropine sulphate). Regions in which the protoplasmic rate is low are more adversely affected than those in which it is high, and which have a greater power of acclimatisation. The result of such treatment in these fish is that the heads are much larger than normal.

These cases fall strictly into line with those in which Child (1924, 1925) was able to alter the size of the head (or polyp) in *Planaria*, *Trochosphere* larvae and *Tubularia* by controlling the relative rate of protoplasmic activity at the top of the apico-basal axial gradient.

(iv) Once the plane of the bilateral symmetry has been fixed, the organs tend to form in relation to their appropriate gradients. This is obvious in the case of the lateral line, but the nerve tube is of greatest importance in this connection. In Ruud and Spemann's (1923) half-gastrulae when the cut coincides with the plane of bilateral symmetry, the cut edges approximate to one another; with the result that the presumptive nerve-tube material no longer lies in a straight line but is bent out of shape and deflected to one side. If the organiser paid no attention to gradients in the material on which it works, the nerve tube should arise straight in front of it. As a matter of fact, it follows the curve which the presumptive material has been forced to make. The organiser therefore works on tissues whose fates are in part the result of the gradient system, and this is why Goerttler (1925) was able to get a certain amount of differentiation of the nerve tube after removing the organiser, and Dürken (1925 *b* and 1926) was able to get pieces of *blastulae* of *Rana fusca* to differentiate. But it must be remembered that the organiser had already long before determined the medio-lateral gradients and the plane of bilateral symmetry. There is danger of grave confusion if it is not realised that the pre-organiser and organiser exercises two functions:

(i) Determination of the plane of bilateral symmetry and localisation of future potencies with reference to this plane and to the egg axis, *i.e.* giving longitude to the egg in addition to the latitude which it already has.

(ii) Invagination as gut roof and evoking the appropriate response from the overlying previously gradiented material. There must therefore be a progressive development of the functions of the organiser.

When an organiser is grafted into the flank of another embryo, both these functions are exercised, and it must be noted that in regard to the former there must be a certain amount of interference in respect of the host's own organiser of the region in which the organiser is planted.

This view of the conditions in the newt's egg lessens the differences between it

and the observed results in the frog's egg. There is however no need to be astonished at the fact that in the latter the gradienting determinative processes appear to act faster than in the former.

Again it may be emphasised that in the determination of regions in a gradient system, it is not the *absolute* rate of activity but the *relative* which is of importance. In other words there must be a "potential difference" of a certain order between regions of high and low rates, with, presumably "epistatic minima."

Coupled with the determination of a region to differentiate into a certain tissue is the loss of potency of other regions to differentiate into that same tissue. This is reflected in the loss of power to form an organ by tissues at increasing distances from the site of that organ. Especially well does this appear in the case of the power of head and trunk tissue to form lens, heart and gills in *Bombinator*.

The processes of chemo-differentiation proceed at different rates in different species. So may be explained the fact that in *Rana esculenta* the lens is already determined at the neural fold stage, while in *Rana fusca* it is not determined until much later. That which determines the lens in *Rana esculenta* must be situated in the rudiment of the eye, and it must persist until the eye touches the epidermis. So it can be understood how the eye of *esculenta* can induce lens formation from strange skin of *Bufo* and *Bombinator*, and how the lens normally arises in *Rana fusca*. Another example of the different speeds at which the gradienting processes work is to be found in the case of the determination of the antero-posterior axis of the limb bud in *Amblystoma punctatum*, *A. tigrinum* and *Triton taeniatus*.

By persistence of the original determining influence, the stage of dependent differentiation often overlaps that of self-differentiation. This it is which simulates "double assurance" and which explains such cases as that of the eye of *Rana esculenta* and its lens-inducing properties.

Owing to these developmental variations, there may be danger in attributing phylogenetic significance to ontogenetic details.

It is to be hoped that future work will not neglect the nature of the transitions from dependent to self-differentiation, and the parts played by physical and chemical processes. Child (1924) has made it probable that the very first differentiation of all is of a dynamic nature, based on transmission of excitation and resulting in "Axiation." When the axiate pattern has been set up, chemical differences may arise between different regions. Stress has already been laid on the difference between chemical (histological) and mechanical (morphological) differentiation. Chemo-differentiated tissue is an "organ-forming substance," such as Conklin (1924) has demonstrated in the ascidian *Styela* by centrifuging experiments. Duesberg (1926) has shown that some of these organ-forming substances are mitochondria. In the frog, however, Jenkinson (1914) showed that normal morphological differentiation could ensue even if the yolk, fat and protoplasm of the egg were quite considerably disarranged. Thus the brain could be normally formed although it contained much more than the normal quantity of fat. Too great a disturbance of course prevents development. Yolk and fat are therefore not specific organ-forming substances but raw materials.

It is obvious that one cannot speak of the "embryo-in-the-rough" until after the stage of chemo-differentiation has started. From that time on, development proceeds largely controlled by internal factors, but always limited by external temperature, humidity, oxygen, osmotic pressure, and later on, function.

But the egg only becomes the embryo-in-the-rough as a result of the impinging on it of two external stimuli. The first evokes the capacity of the egg to become radially symmetrical about any axis. The second brings out its capacity to become bilaterally symmetrical in one of an infinity of planes passing through that axis. Thereafter differentiation arises. At last the Preformation-Epigenesis question has been answered: "Heredity does not account for the individual, but merely for the potentialities some of which are realised in the individual." These potentialities are realised as a result of external stimuli (Epigenesis). Of Preformation in the old sense of spatial prearrangement of already differentiated materials it is of course impossible to speak. The only predetermination which exists concerns the potentialities just mentioned and ensures that if they are realised at all, the resulting organism shall belong to the same species as its parents.

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