Early Embryonic Differentiation.

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THE last general work in English on the subject of experimental embryology was that by the late J. W. Jenkinson, published in 1909.¹ Since then, our knowledge of the subject has advanced enormously, not only in detail but also in principle, chiefly owing to the researches of the schools of Spemann in Germany, Harrison in Yale, and Child in Chicago, and of the workers on tissue culture and endocrinology. No connected account of the whole subject has, however, appeared. (That by Dürken,² in German, 1919, although interesting, takes scarcely any account of the British and American literature.) No excuse is, therefore, offered for directing attention here to some of the important recent discoveries concerning embryological differentiation, and to a possible scheme for their theoretical explanation.

The Amphibia have been the most fruitful material, in some respects the frogs, in others the newts: and we will take them as our type. According to our present knowledge, three fundamentally different processes, essentially consecutive although here and there overlapping, are to be distinguished in Amphibian development up to hatching. (1) Division and re-arrangement of pre-existing material and structure (up to the end of germ-layer formation). (2) Primary or non-functional differentiation, including (a) determination and (b) differentiation of the primordia of organs. (3) Secondary or functional differentiation.

Up to 1910, the principal achievements of experimental embryology could be summed up thus: first, that nuclear division in early development was not differential, the nuclei of the embryo all being equivalent; secondly, that (usually and primitively) most fragments of the germ before the onset of gastrulation could, if of sufficient size, regulate themselves to produce a whole embryo or an approximation to it; thirdly, that in a few cases definite "organ-forming stuffs" existed in the fertilised egg—visibly-differentiated regions causally correlated with the development of certain organs; and fourthly, that Roux's doctrine of the "Struggle of the Parts" was valid in later stages. These we assume in what follows.

The Amphibian egg, like most eggs, starts with a definite if simple organisation. It is stratified from animal to vegetative pole as regards the proportion of cytoplasm and yolk, and the nucleus is excentric. This visible polarity or material gradient appears to be the result of a pre-existing metabolic gradient (Child³); the rate of metabolic activity (as tested by rate of dying in weak toxic solutions, which in more favourable material has been shown to run parallel with rate of oxidation, carbon dioxide production, and degree of electro-negativity⁴) is greatest at the animal pole, and diminishes gradually to the vegetative. This permits of the accumulation of inert substances like yolk towards the vegetative pole, while they are more readily oxidised towards the animal pole.

The front of the head is predetermined to form near the animal pole, the tail to grow out near the vegetative pole. In addition to this major (polar) axis, and consequent radial symmetry, which is all that the egg possesses before fertilisation, Brachet,⁵ Spemann,⁵

Jenkinson,⁶ and others have shown that the unsegmented but fertilised ovum already possesses bilateral symmetry, since the region of the dorsal lip, which marks the future dorsal side of the embryo, is already fixed. It is usually stated that this is determined by the place of entry of the spermatozoon. Although this is undoubtedly a predominant factor, yet Jenkinson⁶ showed that the direction of incident light may play a part. In any event, not only the bilaterality of the future organism, but also, as we shall see, the region which initiates all subsequent differentiation, is determined, at or about the time of fertilisation, by agencies external to the ovum.

The power of regulation is possessed by fragments of the germ during the segmentation stage, but only if (a) the division runs more or less along the polar axis, (b) if the fragment includes some or all of this future dorsal-lip area. If the division cuts the dorsal-lip area in two, both halves will regulate, and twin embryos are the result; but if all the area is in one half, only this half will form an embryo, the other portion forming a vesicle, fully segmented, and with the three germ-layers, but no differentiation of organs.

Spemann⁷ has proved that the dorsal-lip region is a *differentiator* (or "organisator" as he styled it) by grafting some of it to another embryo of a different species, where it can still be recognised by its colour. Even if grafted into an area which should only have formed flank ectoderm, it causes the host tissues between it and the animal pole to form the "primary axial organs" (neural plate, notochord, and somites). It is noteworthy that the dorsal-lip region is the region of most active cell-division and presumably of most active metabolism; it may well be that it is the stimulus of its activity as such which causes the neighbouring cells to differentiate, as would be expected on Child's theory.

Brachet⁵ has recently shown that the dorsal-lip region exerts its formative effect both anteriorly and posteriorly. If continuity between the dorsal lip and e.g. posterior region is interrupted, no differentiation of axial organs (notochord, nerve cord, and somites) will occur behind the interruption. The influence appears to demand contact for its spread (cf. optic cup and lens—see below). In spite of absence of axial organs, other organs such as Wolffian duct and heart will be normally differentiated. This can be explained on Child's theories, since they form at low levels of the gradient; provided some of the dorsal region is intact, sufficient "potential difference" exists to determine these low-level organs.

It is important to note that the influence of "high-level" active organs on the morphogenesis of other organs is not new, but is well known in regeneration. Child³ has shown (and the experiment can be easily repeated) that the formation of the pharynx in a piece cut from a Planarian is only possible in the presence either of a head or of other tissues normally anterior to the pharynx.

In our period of primary differentiation the first step is the irreversible decision as to which region shall produce which organ. This occurs some time before

any visible organ-rudiments appear. Thus it is important to distinguish the invisible *determination* from the visible *differentiation* which is its consequence. For example, Spemann⁷ has proved that in the early neural plate stage, before the least trace of optic vesicle is to be seen, not only is there predetermination of the eye as a whole, but also of the separate areas for optic stalk, retina, and tapetal layer, each in definite quantity. Bulbar, ventricular, atrial, and sinus substances are similarly predetermined before the heart appears.

We may best sum this stage up by saying that primary differentiation initiates a period in which the organism is essentially a *chemical mosaic* of qualitatively unlike regions. Regeneration (except, in certain cases, of one of two paired organs) during this period is impossible. If a gastrula be cut into two, each half forms only those organs which it would have formed as part of a whole; if a piece of the future brain region is cut out in the neural plate stage, and then grafted back in reversed position, its different parts still produce the structures they would have produced if in normal position. Some *form-regulation* of separate organs is possible (although no approach to the normal form of the whole organism); gastrula-halves round off; both halves of a bisected optic-cup region form miniature eyes, but each chemically differentiated region is separate and irreplaceable. Adult newts, for example, may regenerate disarticulated limbs, even together with their limb-girdles; but a newt embryo from which one of the small "limb-areas" of ecto- and mesoderm has been removed will remain permanently limbless.⁸

Form-regulation is thus largely a matter of physical factors; but differentiation proper depends upon more important chemical differences. This complete lack of regeneration, and the behaviour of the organism during a whole period as a mosaic of independent regions, instead of, as in later periods, a system of interrelated organs, is not only interesting in itself, but also of great theoretical importance in view of the assertions of Driesch,⁹ Haldane,¹⁰ and others that regulation is the fundamental property *par excellence* of organisms.

Even in higher forms the same holds good. The head region of a 24-hour chick grafted on to the membrane of a 7-day egg will differentiate essentially as it would have done normally, and without regeneration,¹¹ and the early rudiment of the metanephros of an older chick, excised and similarly treated, will differentiate in quite a characteristic fashion in the solitude of the chorio-allantois.¹²

Spemann found early gastrulation to mark the onset of chemical determination; it is worth noting that many workers using, *e.g.*, echinoderm and teleost material, and employing quite different methods, have concluded that gastrulation is the time at which the chromosomes first begin to exert a detailed chemical action.¹³ It might be well to emphasise this aspect of the matter by using some such term as *chemo-differentiation* for what I have called primary differentiation.

The various irreversibly determined regions appear to differ from each other by the presence of qualitatively different substances, the concentration of which is

highest in the centre of the region and diminishes towards its margin.

If it is asked how we can imagine the process as originating, the answer must, I think, follow some such lines as these. During gastrulation every portion of the germ has a definite relation to the germ's system of metabolic gradients. Of these, there are (1) the primary apico-basal gradient; (2) the dorso-ventral, determined by the fact that the active dorsal-lip region is on one side of the organism (as a matter of fact, actively dividing cells pass down the outer limb of the dorsal lip from equator to vegetative pole, others are invaginated and extend on its inner limb almost to the animal pole, so that the whole dorsal region is one of activity).

Both these gradients extend both on the surface and internally, and together constitute a three-dimensional system of "gradient co-ordinates." Every portion of the germ, therefore, has its own rate of activity corresponding to its position in the existing co-ordinate system, and its own characteristic proportions of yolk, glycogen, cytoplasm, etc., depending on the previous effect of the apico-basal gradient during the growth of the egg. When the "differentiator," the dorsal lip, exerts its admittedly as yet unexplained, though not unparalleled, action of initiating differentiation, every region of the germ is in a different condition from every other. The substrate is different from place to place: the result, therefore, also differs.

In addition, one region may have a definite chemical effect on another, as, for example, when the optic cup induces lens formation in the epidermis (Lewis, Spemann, Bell¹⁴).

It appears that in vertebrates the position of a region in the gradient co-ordinate system is of more importance than the proportions of primary materials it contains. Jenkinson,¹⁵ by centrifuging fertilised frog's eggs, found that qualitative differentiation would still proceed even when the normal proportions of fat, protoplasm, and yolk were much altered. When they were too much altered, development was subnormal, and finally failed to proceed. In much the same way, it would be impossible to build a conservatory if the proportion of steel or wood to glass were too much reduced. The substances which can be distinguished in the unsegmented ovum of a vertebrate are thus merely raw materials. The specific organ-forming materials found elsewhere, *e.g.* in Dentalium,¹⁶ are exceptional specialisations. They represent *precocious* chemo-determination, but differ from the chemically defined regions of the late gastrula of vertebrates in not being formed in their definitive positions, but undergoing a complicated migration. The results of centrifuging invertebrate eggs also bear out the view that the visibly differentiated substances are only the raw materials, the expression of a more ultimate organisation—presumably the living gradient-system—which is relatively unaffected by the process of centrifuging.¹⁷

Of course, all the organs do not appear simultaneously; but each at its apparition seems to pass through the same stages: (1) indifferent or at least pluripotent material; (2) chemical determination; (3) visible differentiation. For the first origin of late-, as for

early-forming organs, the gradient co-ordinate system appears to be of paramount importance. For example, Harrison¹⁸ has shown that the limb-area before the appearance of the limb-bud is already "polarised" by the main apico-basal (now antero-posterior) gradient of the larva. But its dorso-ventral gradient is not yet irrevocably determined; and if it is grafted in upside-down, the existing dorso-ventral gradient of the whole organism determines the definitive dorso-ventral differentiation of the limb.

An even more striking example is found in another of Harrison's experiments.¹⁹ He grafted the front portion of an embryo of one frog species on to the dorsum of a whole embryo of a lighter-coloured species. When the lateral-line system of the dark graft began to grow out, it could be followed on the light skin of the host. When it had nearly reached the centre of the flank it began to curve, and finally bent right round and grew backward in the proper position—proper relative to the host's organisation. Apparently this means that it is adjusted to a particular "level" in the dorso-ventral gradient, and to an apico-basal direction of growth.

Early histological differentiation appears for the most part to be a direct consequence of the original chemo-determination. But the *arrangement* of the cells and cell-processes is another matter. In great measure this is determined by function, and falls into the period of secondary differentiation; but in one very important case at least—that of the nervous system—it seems to depend largely upon the gradients. In tissue culture, embryonic neuroblasts throw out axons and dendrites in normal fashion, but in random directions. If, however, a weak electric current is passed through the culture medium, all the axons are oriented with respect to the current, as Ingvar's experiments have shown.²⁰

Now Child and his pupils, especially Hyman,⁴ have established that the gradient in electric potential, originally discovered by Morgan in the earthworm, is a constant feature of organisms, and may properly be considered as one manifestation of the gradient in metabolism. Thus in the undifferentiated nerve-tube there will already exist a gradient in potential from head to tail; and this should cause a growth of axons posteriorly within the nervous system. Once these neurons are differentiated, then, as Kappers²¹ has shown, their stimulation will be accompanied by a current in the axon, and this in its turn will determine the growth of the axons of neighbouring neuroblasts at right angles to the existing axons. Thus we get some clue as to how axons should grow down the cord and out at right angles to it, and so (and in Kappers' other principles of neurobiotaxis) we get our first clue as to the mechanism by which the architecture of the nervous system is established in ontogeny.

These processes, which in Amphibia are unaccompanied by growth, bring us to the stage at which the organs are ready to begin their specific functions. Once this starts, a new period begins—a period which converts the chemical mosaic into a whole with inter-related parts, by means of functional response, nervous control, and humoral regulation. This, however, cannot be discussed within the limits of the present article.

It will be seen that the discovery of a "differentiator" and of the gradient co-ordinate system enables us for the first time to give a coherent formal account (however imperfect in detail) of the early stages of development. In so doing we sail clear of the difficulty, which has beset so many minds, of understanding how differentiation can be compatible with absence of qualitative nuclear division. Loeb, in his book "The Organism as a Whole,"²² was driven to assume that the chromosomal (Mendelian) genes were only responsible for minor characters, the main course of development being determined by the ovum, which, owing to its assumed possession of localised organ-forming stuffs (supposedly proteins), was to be regarded as "the embryo in the rough."

It is now seen that the germ cannot be held to become "the embryo in the rough" until chemo-differentiation has started. After this moment, organ-forming substances are all-important, but in most unfertilised eggs scarcely exist. The production of these organ-forming substances depends upon the varying interaction of differentiator and genes in regions of various activity. The differential which determines the variation of activity is the system of metabolic gradients, which, although definitely organised, is very far from constituting the egg an embryo, however much in the rough. One of the two main gradients is determined at or after fertilisation by agencies external to the ovum. The other is determined in the unfertilised egg; but from analogy with other forms, it is to be expected that this too will be found to have been earlier determined by agencies external to the oocyte (position of the oocyte in the germinal epithelium, blood-supply, etc.²³).

There is thus no reason for calling in question the efficacy of the chromosomes (Mendelian factors) as regulators of development, whether individual, specific, or phyletic. In the absence of evidence to the contrary, and in consonance with all our positive knowledge, it is simplest to assume that they provide both the necessary complexity and the necessary specificity for development, while the two main gradients provide the difference between parts of the germ necessary for the start of localised qualitative differentiation, and the activity of the dorsal lip the energy needed to set the process in action.

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