OXIDATION-REDUCTION PATTERNS IN AMPHIBIAN AND TELEOST DEVELOPMENT

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The problem of amphibian developmental pattern has been attacked by many investigators in widely different ways, but the question of the essential characteristics of this pattern and of vertebrate developmental pattern in general is still under discussion. The following briefly presented data, giving evidence of patterns of enzyme activity, are concerned with this question.

Methods.—Intracellular reduction and reoxidation of methylene blue and Janus green and formation, reduction and reoxidation of indophenol in living intact developmental stages of a urodele, Triturus, and a teleost, Oryzias latipes, show the presence of definite oxidation-reduction patterns, characteristic for particular developmental stages, and undergoing definite changes in the course of development.* Lightly pigmented and occasionally occurring unpigmented egg masses of Triturus permit direct observation of these patterns. Triturus embryos remaining within the vitelline membrane after removal of the jelly stain very slowly with oxidized methylene blue, but with decrease of free oxygen in the solution by addition of a minute amount of sodium hydrosulphite the dye, reduced to the colorless “leucobase,” penetrates both vitelline membrane and embryo almost at once, and with increase of oxygen recoloration occurs.

The teleost embryos within the chorion stain with oxidized methylene blue, but the chorion stains so rapidly and deeply that it may become difficult to see the embryonic patterns. Oxidized Janus green also stains through the chorion but when reduced to the colorless form by hydrosulphite it penetrates more rapidly and reoxidizes to red.

Intracellular dye reduction is brought about in both amphibian and teleost by oxygen decrease, either rapidly by hydrosulphite, or more slowly by oxygen uptake of embryos sealed in small volume of dye solution. On increase of oxygen after reduction reoxidation of intracellular dye with
recoloration occurs in a definite differential pattern. In the extremely small amounts used, sodium hydrosulphite is not appreciably toxic except perhaps after often repeated additions to the dye solution. Reduction and reoxidation of methylene blue can be repeated several times without affecting further development.

Intracellular formation of indophenol, deep blue in oxidized form, from dimethylparaphenylenediamine (para-aminodimethylaniline) and α-naphthol, catalyzed by an oxidase, is finally toxic or lethal, but with low concentrations of reagents the intracellular reaction pattern becomes clearly visible before toxic effects appear. The reaction is of little value for intact earlier embryos of Triturus because it is extremely slow or inappreciable, but it occurs readily in cells adjoining cut or torn surfaces of isolated pieces in Holtfreter solution or locally injured regions of these stages, and after closure of the neural tube and in young animals after hatching it takes place relatively rapidly. Teleost embryos within the chorion show the reaction at all stages, but more rapidly later than earlier, and much more rapidly in regions or adjoining surfaces where relations of cells have been disturbed by cutting, tearing or isolation of pieces, than in intact parts. After intracellular formation indophenol is reduced to the colorless form and reoxidized with recoloration in the same ways as methylene blue.†

In living amphibian and teleost material in good condition the gradient patterns shown by the indophenol reaction and by reduction of indophenol, Janus green and methylene blue coincide in direction and recoloration on reoxidation progresses in the reverse direction.

*Pattern in Triturus Development.*—In early cleavage reduction of methylene blue and Janus green progresses from the apical (animal) region, more rapidly on one side, presumably the dorsal side. In blastula stages the gradient from the apical region is present and reduction also spreads from an area on the presumably dorsal side. As soon as the earliest stages of invagination permit identification of dorsal and ventral sides with certainty the dorsal lip of the early blastopore appears as the most rapidly reducing region of the embryo and reduction progresses anteriorly and somewhat laterally from it. This dorsal area of rapid reduction is clearly distinguishable to the naked eye and reappears in successive reductions until overstaining or hydrosulphite produces toxic effects. It evidently coincides approximately with the dorsal inductor region but is without distinct boundary, the decrease in reduction toward its anterior and lateral border being gradual. In these early gastrula stages the reduction gradient from the apical region is slight but still visible when pigmentation is not too deep. Dorsally it meets the gradient from the dorsal lip above the equator. The reduction pattern in the dorsal inductor region persists during gastrulation but in later gastrula stages usually appears somewhat less extensive. As the blastopore progresses from crescentic to circular outline,
that is, as invagination extends laterally and ventrally, reduction usually appears somewhat more rapid in lateral and ventral lips, but the gradient does not extend far laterally and ventrally from the lip, and reduction is never as rapid as in the dorsal lip. After closure of the blastopore a small area, chiefly anterior to the blastopore region, still shows relatively rapid reduction for a time.

With beginning of neurulation a further change in pattern appears. Even before the neural plate is distinctly visible reduction becomes more rapid in its anterior region than elsewhere and progresses posteriorly from it. As the neural folds appear they become regions of rapid reduction, also progressing posteriorly. Reduction outside the neural plate is much less rapid and progresses posteriorly and ventrally. In later neurula stages and after closure of the neural tube reduction progresses from the anterior head region posteriorly, rapidly in the dorsal region and posteroventrally and much less rapidly in lateral regions. Early stages of the tail bud are associated with a new region of more rapid reduction and with outgrowth of the bud this becomes a reduction gradient with high end at the tip.

Reduction of methylene blue, Janus green and indophenol and the indophenol reaction become more rapid in early bud stages of the fore leg than in surrounding lateral regions. With outgrowth of the leg the almost radial gradient pattern of the bud becomes longitudinal in consequence of differential growth, with rates of reaction and reduction decreasing from the tip. In the outgrowing leg rates of reaction and reduction also decrease from the anterodorsal to the posteroventral side, that is, the developing leg apparently retains the anterodorsal-posteroventral gradient of the body. Since a longitudinal gradient pattern is also present in it, it possesses pattern in three dimensions long before morphological differentiation is evident and probably from the beginning of its outgrowth.

In outgrowing gill filaments and balancer rates of reduction and of indophenol reaction decrease from the tip. In the gill filament and filament complex of later stages reduction is more rapid on the arterial than on the venous side, probably because of lower oxygen content of blood coming to the gill.

In general, regions which reduce more rapidly show recoloration on reoxidation less rapidly than others, that is, reoxidation gradients are the reverse of reduction gradients, but all gradient patterns may be partially or wholly reversed or obliterated by differential toxic effects of overstaining, indophenol, long continued low oxygen or hydrosulphite.

Pattern in Oryzias Development.—With this form intracellular formation, reduction and reoxidation of indophenol were used to a greater extent than reduction and reoxidation of methylene blue and Janus green, but all give the same results. In early cleavage and blastoderm stages the central region of the blastoderm, where cell division is more rapid, seems to show
indophenol reaction and reduction somewhat more rapidly than the margins, but the difference is at best slight. Before invagination begins one side of the blastoderm reacts and reduces more rapidly than other parts. At the earliest stages of invagination it becomes evident that this is the side on which invagination begins, and the region of rapid reduction and reaction corresponds to the region of rapid reduction indicating the dorsal inductor in *Triturus*. In slightly later stages of invagination this dorsal inductor region becomes still more clearly distinguishable with rate of reduction and reaction decreasing anteriorly from the lip of the blastopore and laterally from the prospective median region of the embryo. With development of the germ ring around the yolk, its lateral and ventral regions show slightly more rapid reduction and reaction than regions anterior to them.

As the embryonic area or "shield" forms, reduction and reaction become increasingly rapid in its anterior region where the anterior end of the embryo will develop. At this stage there are two opposed longitudinal gradients in the embryo, one from the dorsal lip, the other from the anterior embryonic region. Rates of reduction and reaction also decrease from the median region laterally. With progress of germ ring over the yolk and closure of the blastopore, the posterior gradient becomes less distinct and disappears, and reduction and reaction progress from the anterior end posteriorly, with a short gradient in the opposite direction as the tail develops. This anteroposterior gradient, together with progress of reduction and reaction from the dorsal region ventrally in head and body, persists in later embryonic stages. The dorsiventral gradient shows the greatest differences in the head region and appears even in the developing eyes. There reduction and indophenol reaction decrease in rate ventrally and slightly posteriorly from the dorsal and slightly anterior region and pigment development follows the same course. Evidently the eye, as a lateral outgrowth, like the leg, retains the general gradient pattern of the body. If this is the case in the urodele eye, it is probably a factor in determining lens regeneration from the dorsal margin of the iris.

In *Oryzias*, as in *Triturus*, cells which have been disturbed in their relations by cutting or tearing the embryo during or after removal from the chorion in a Ringer solution, even in stages before gastrulation, and isolated cell groups and cells show more rapid indophenol reaction than intact embryos or parts, suggesting that oxidase activity may have been increased by the injury. Differences in rate of reduction are more variable because torn or cut regions and isolated cell groups react so much more rapidly than intact embryos or parts that they become deeply colored and may be damaged while reaction is still very slight in the undisturbed cells. With intact embryos and isolated parts in separate solutions there is no certainty as regards intracellular concentrations of indophenol. However,
with more or less similar, apparently non-toxic concentrations, as indicated by color, a far from trustworthy indicator in cell masses of different thickness when viewed by transmitted light, isolated cell groups and cells, and cells adjoining torn or cut surfaces often reduce more rapidly than intact parts and reoxidize more slowly.

Discussion.—The indophenol reaction and intracellular reduction and reoxidation of indophenol, methylene blue and Janus green in living intact individuals all show the presence of definite gradient patterns in early development of amphibian and teleost. The more general features of these patterns in the two animals are essentially similar as regards their relations to embryonic axes and prospective regions. The patterns are present long before there is any evidence of morphological differentiation and undergo definite changes in the course of development, with localizations of regions of accelerated reaction and reduction which later induce or develop particular organ systems. All these characteristics indicate that these patterns are expressions of essential physiological factors of development, and that different specific systems of activities and conditions and qualitative differentiations develop from primarily quantitative gradient patterns. The early outgrowing amphibian leg undoubtedly differs specifically in some way from early gill filament and balancer, and all of these differ from the gastrula, but a quantitative gradient pattern appears in all. The local differences characterizing leg, gill filament and balancer originate within, and in definite relation to, the gastrula pattern, and the local differences within leg, gill filament and balancer arise within, and in definite relation to, the gradient patterns of early stages of these organ systems.

Whether regional differences in reduction, reoxidation and indophenol reaction do or do not coincide with regional differences in oxygen uptake and CO₂ production, and whether the assumption is or is not justified that respiration of small isolated fragments of embryos remains the same as when they were parts of the intact individual, it appears evident that respiratory determinations on small isolated fragments do not by any means tell the whole story as regards physiological developmental patterns. Moreover, the indophenol reaction, and less certainly, reduction and reoxidation, indicate that physiological condition may be considerably altered in small isolated pieces of embryos.

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† Using low concentrations of reagents, it has been possible to show presence of definite indophenol oxidation-reduction patterns in other living intact organisms. Such patterns in the echinoid, Dendraster, have already been described (Proc. Nat. Acad. Sci., 27, 523 (1941). Data on ciliate Protozoa, and annelid, Nais paraguayensis, development of the starfish, Patiria, the ascidian, Clavellina, and ovaries of Drosophila are still unpublished.