

The Significance of Vertebrate Metamorphosis

A life cycle is circular; and its completion may involve two opposed metamorphoses, biochemical components of which pervade the vertebrate kingdom.

George Wald

"Nicodemus saith unto him, How can a man be born when he is old? can he enter the second time into his mother's womb, and be born?

"Jesus answered, Verily, verily, I say unto thee, Except a man be born of water . . ."—John, 3: 4, 5.

One of Thomas Mann's novels begins with the words, "Very deep is the well of the past." This is the well from which all biology is drawn. Physics and chemistry deal with what is; biology with what has become. In biology one is never far from history; and each living organism carries fragments of this history with it, as vestiges and recapitulations, anatomical and, as we shall see, biochemical.

I was led into this subject through the study of visual systems. I shall begin with them since they still provide the main thread of the argument, though by now it branches widely.

What we are concerned with here is an aspect of biology at the molecular level. Like all such developments, it runs closely parallel with earlier arguments based primarily upon anatomy. This is hardly surprising, for the anatomy of an organism is the greatly magnified expression of its chemistry; and the short- and long-term changes in anatomy that con-

stitute the organism's embryogeny and evolution, by the same token, express chemical changes. The biochemist is a biologist whose dissections have reached the molecular level. Far from removing him from biology, this gives him new opportunities to pursue it.

For like reasons, some of the viewpoints and conclusions expressed here (1) were reached long ago by other paths. They will bear this reiteration and the new support that biochemistry brings them.

Primacy of the Spawning Environment

Two kinds of visual system are found in the rods of vertebrate retinas. One is based upon the red visual pigment, rhodopsin, formed by the combination of the protein opsin with retinene, the aldehyde of vitamin A. The other is based upon the purple pigment, porphyropsin, formed from the same type of opsin combined with retinene₂, the aldehyde of vitamin A₂. Retinene₂ and vitamin A₂ differ from retinene and vitamin A only in possessing an added double bond in the ring (Fig. 1) (see 2).

The porphyropsin system was first discovered in fresh-water fishes. Marine fishes and land vertebrates characteristically possess the rhodopsin system (3, 4) (Figs. 2 and 3).

What of the fishes that are neither fresh-water nor marine, but migrate between both environments? It would be well before discussing them to clarify somewhat their biological position.

Most fishes are restricted throughout their lives to narrow ranges of salinity. Such forms are called "stenohaline" and are of two kinds, fresh-water and marine. A much smaller group of fishes can live as adults in a wide range of salinities. They are called "euryhaline," and, again, are of two kinds, anadromous and catadromous, meaning "upstream" and "downstream." These terms refer to the direction of the spawning migrations. Salmon, for example, are typically anadromous forms, coming upstream to spawn, whereas the "fresh-water" eels are catadromous, going downstream to the sea on their spawning migration.

It is probably true, however, that no euryhaline fish has to leave its spawning environment to complete a normal life cycle. Many instances are known in which anadromous fishes remain permanently in fresh water. The same is true of such an anadromous cyclostome

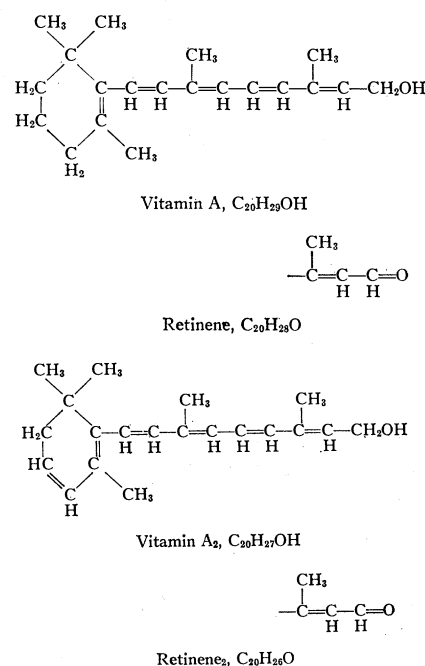


Fig. 1. Structures of vitamin A, retinene, vitamin A₂, and retinene₂.

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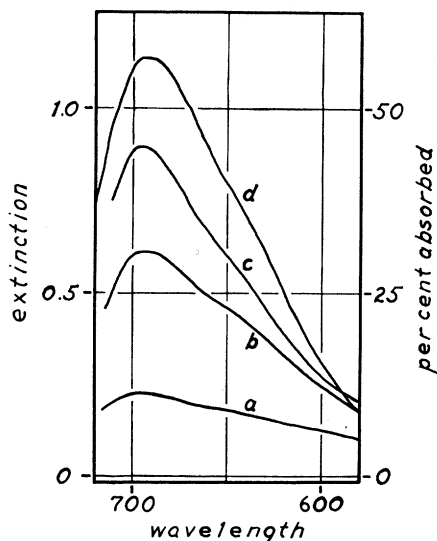


Fig. 2. The vitamin A_2 of the retinas of fresh-water fishes. Antimony chloride tests with extracts of wholly bleached retinas display only the absorption band maximal at 690 to 696 $m\mu$ characteristic of vitamin A_2 . This result has been obtained invariably in about 12 widely distributed species of fresh-water teleost. In almost all cases the visual pigment has also been extracted and this pigment has been found to be porphyropsin, with λ_{max} about 522 $m\mu$. [From Wald (3)]

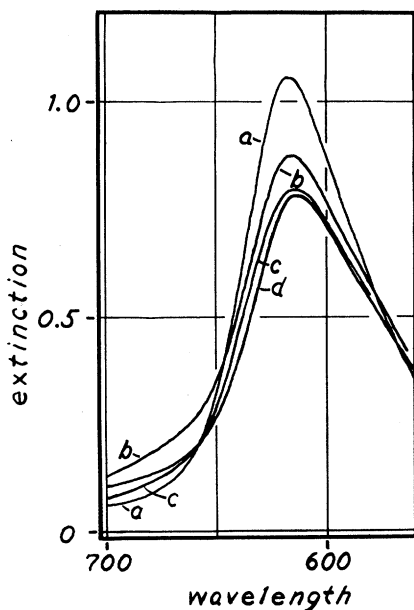


Fig. 3. The vitamin A_1 of the retinas of marine fishes. Spectra of the antimony chloride tests with extracts of bleached retinas reveal the λ_{max} at 615 to 620 $m\mu$ characteristic of vitamin A_1 . This result has been obtained with a great variety of bony, and a few elasmobranch, fishes and is characteristic also of land vertebrates. Two wrasse fishes (*Labridae*), however, the cunner and tautog, though wholly marine, are exceptional in having a predominance of vitamin A_2 in their retinas. [From Wald (3)]

as the sea lamprey, which has recently colonized the Great Lakes and virtually destroyed the fresh-water fisheries there.

So far as we know, the spawning environment is always fixed. The eggs, the sperms, or the embryos, perhaps sometimes all three, are stenohaline. Euryhalinity develops later in life and permits, though does not compel, these animals to migrate to the other environment. Migration is only a potentiality, which some of these forms exploit regularly and others rarely. The salmonids are essentially fresh-water fishes with the privilege of going to sea as adults; the fresh-water eels are marine fishes with the capacity of coming as adults into fresh water.

The significant biological statement concerning such fishes is not that they migrate but that, being fixed in spawning environment, they are euryhaline as adults. I should like on this basis to redefine the terms applied to them. An anadromous fish is a euryhaline form which spawns in fresh water; a catadromous fish, one which spawns in the sea (3).

On examining the visual systems of several genera of salmon, I found that all of them possess mixtures of the rhodopsin and porphyropsin systems, yet primarily the latter, characteristic of the spawning environment. Conversely, the American fresh-water eel possesses a mixture of both visual pigments, in which rhodopsin—again the spawning type—predominates (Fig. 4) (5). Certain other anadromous fishes—alewife, white perch—possess porphyropsin almost alone (Fig. 5). All the euryhaline fishes examined follow a simple rule: all of them possess, either predominantly or exclusively, the type of visual system characteristically associated with the spawning environment (3, 6).

To a first approximation these patterns are genetic and independent of the immediate environment. The salmonids which were found to possess mixtures of both visual systems had spent their entire lives in fresh water. Alewives just in from the sea on their spawning migration possess porphyropsin almost exclusively. Most striking of all, the cunner and tautog, members of the wholly marine family of *Labridae*, the wrasse fishes, possess porphyropsin; this is the only type of marine fish yet known to do so (3, 6).

Since the distribution of visual systems among fishes is genetic, one may ask whether it fits into some evolutionary

pattern. Many paleontologists are convinced that the vertebrate stock originated in fresh water. It is from such fresh-water ancestors that our fresh-water fishes were ultimately derived (7). The observation that these animals characteristically have the porphyropsin system suggests that this may have been the ancestral vertebrate type. Subsequently vertebrates undertook two great evolutionary migrations, one into the sea, the other to land. Both led them to the use of rhodopsin in rod vision, for this is the pigment we find characteristically in marine fishes and land vertebrates. The euryhaline fishes are intermediate between fresh-water and marine forms both in life history and in the composition of their visual systems. In this regard one can arrange the fishes in such an ordered sequence as that shown in Fig. 6.

Euryhaline Fishes and Amphibia

One can hardly develop such an argument as outlined above without raising questions regarding the amphibia. These

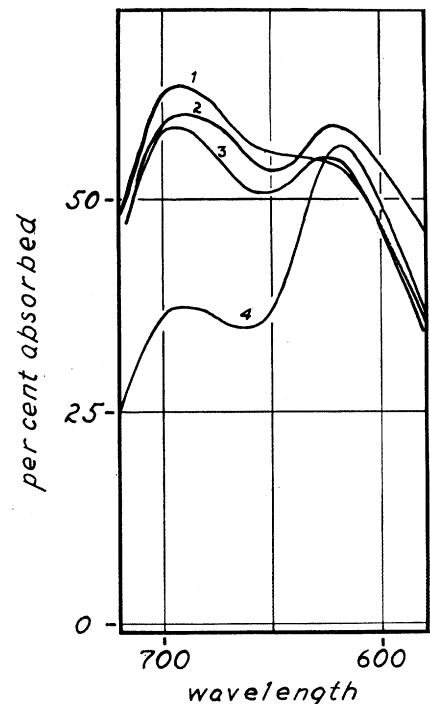


Fig. 4. Retinal vitamins A in euryhaline fishes. Spectra of antimony chloride tests with extracts of bleached retinas from (1) chinook salmon, (2) rainbow trout, (3) brook trout, and (4) the American "fresh-water" eel. All these tissues contain both vitamins A_1 and A_2 , the anadromous salmonids a predominance of vitamin A_2 , the catadromous eel a higher proportion of vitamin A_1 . [From Wald (3)]

animals come between fresh-water fishes and land vertebrates, just as the euryhaline fishes do between fresh-water and marine fishes. Most amphibia, like most euryhaline fishes, spawn in fresh water. Indeed, the life cycle of the common frog runs strikingly parallel with that of such an anadromous fish as the salmon. Both originate and go through a larval period in fresh water. Both, after undergoing deep-seated anatomical and physiological changes which can in both be described as metamorphosis, migrate for the growth phase, the salmon to sea, the frog to land. Both return to fresh water at sexual maturity to spawn. What land is to the frog, the sea is to the salmon. *The euryhaline fishes are the amphibia among the fishes.*

In this sense one might speak of almost all amphibia as "anadromous," meaning that they spawn in fresh water and are free, as adults, to go back and forth between fresh water and the land. [One would like to commit further etymological atrocities. The essence of the euryhaline condition is the capacity to migrate, not so much between low and high salt concentrations as between hydrating (fresh-water) and dehydrating (sea and land) conditions. In this sense the amphibia are "euryhaline." Obviously one needs new terms, firmly grounded in ecological essentials rather than in trivialities.] A few amphibia (red-backed and tree salamanders) have developed special devices for living permanently ashore. I know of no "catadromous" amphibian—that is, one that spawns on land and goes through its growth phase in the water. A number of aquatic reptiles, however (alligators, fresh-water snakes, and turtles), fulfill this description nicely.

If these are substantial parallels, and if the spawning environment decides the pattern of visual pigments, then one should expect such an "anadromous" amphibian as the common frog to possess mainly porphyropsin, like a salmon. Yet rhodopsin was originally discovered in the rods of frogs, and for a long period all that we knew of this pigment was learned with frogs.

In this dilemma I turned to a tailed amphibian, with the thought that it might display more primitive properties than the tail-less types. Adults of the common New England spotted newt were found to possess porphyropsin exclusively (8, p. 337). This brought a first amphibian into the same fold with certain anadromous fishes but left the frog

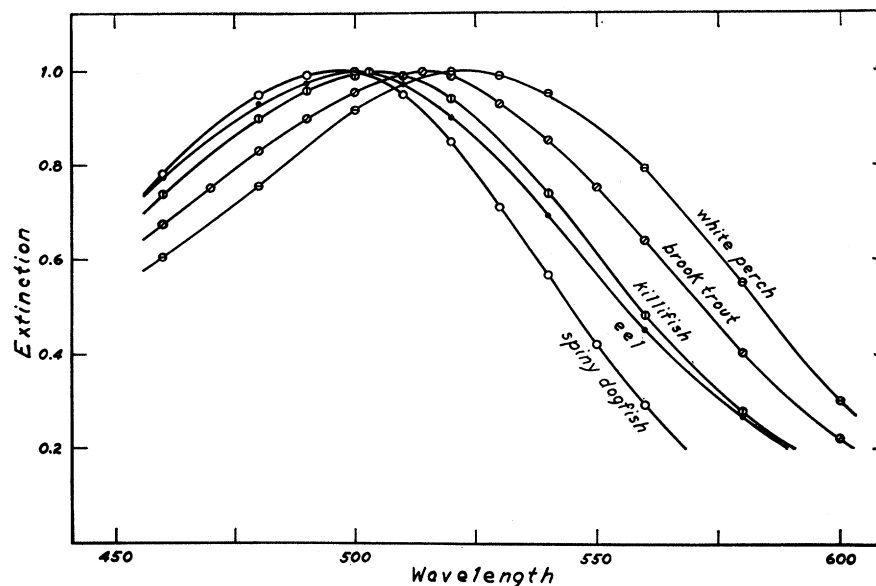


Fig. 5. Spectra of the visual pigments of various fishes, illustrating the transition from an exclusively rhodopsin to an exclusively porphyropsin system, correlated with salinity relations. The permanently marine dogfish possesses rhodopsin alone (λ_{max} about 497 m μ); the catadromous eel and brackish-water killifish, predominantly rhodopsin; the anadromous brook trout, predominantly porphyropsin; and the anadromous white perch, wholly porphyropsin (λ_{max} about 522 m μ). [From Wald (6)]

in a more aberrant position than ever.

On examining bullfrogs in metamorphosis, however, I found that tadpoles just entering the metamorphic crisis possess porphyropsin almost entirely, whereas newly emerged frogs have changed almost entirely to rhodopsin (9). The anatomical metamorphosis, which in this species takes about three weeks, is accompanied by this biochemical metamorphosis of visual systems. The bullfrog enters metamorphosis with porphyropsin, like a fresh-water fish, and emerges with rhodopsin, like a land vertebrate (10) (Fig. 7).

Individual amphibia, therefore, like euryhaline fishes, may display both types of visual system. It seemed for a time that one difference between both groups might be that in euryhaline fishes the patterns of visual system are fixed, whereas in amphibia they change abruptly with metamorphosis. I shall have more to say of this later.

Biochemistry of Metamorphosis

At the time the metamorphosis of visual systems was discovered in the bullfrog, another such change in the same species had already been described. McCutcheon (11) had found that the properties of hemoglobin in this animal change markedly at metamorphosis. The

oxygen equilibrium curve of hemoglobin, measured at one temperature and pH, goes through a remarkable transition between tadpoles and adults. The hemoglobin of tadpoles has a high affinity for oxygen, and it seemed from McCutcheon's measurements that the shape of its oxygen equilibrium curve might be hyperbolic, whereas the hemoglobin of young adults has a relatively low affinity for oxygen, and its equilibrium curve is distinctly S-shaped.

Riggs (12) reexamined this situation

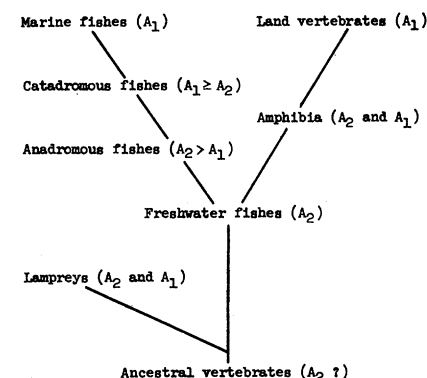


Fig. 6. Distribution of vitamins A_1 and A_2 in vertebrate retinas. The observations, all made on contemporary animals, are here correlated with the present ecology. They may also, however, represent evolutionary sequences, and in that case they convey the suggestion that primitive vertebrate vision was based upon vitamin A_2 .

in our laboratory. He confirmed McCutcheon's finding of a striking loss of oxygen affinity at metamorphosis. He found, however, that the shape of the oxygen equilibrium curve does not alter at metamorphosis; it is equally sigmoid throughout development. He found another important change: tadpole hemoglobin exhibits almost no loss of oxygen

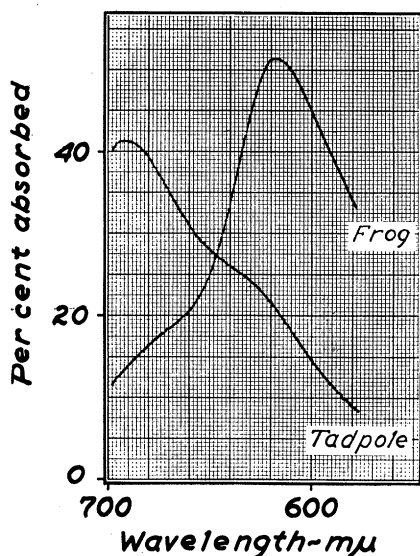


Fig. 7. Biochemical metamorphosis of visual systems in the bullfrog, *Rana catesbiana*. The tadpole just entering the metamorphic crisis has in its retina vitamin A₂ (that is, porphyropsin) with only a trace of vitamin A₁ and rhodopsin, whereas the newly emerged froglet has just the reverse pattern. [From Wald (9)]

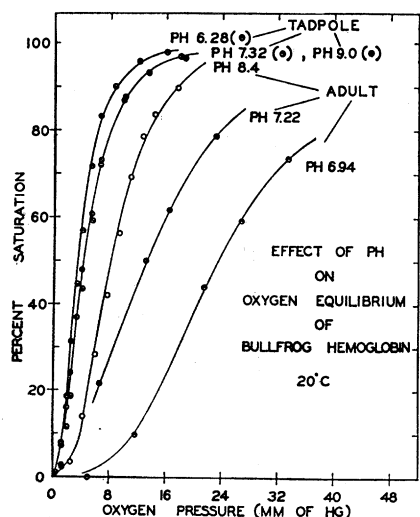


Fig. 8. Biochemical metamorphosis of hemoglobin in the bullfrog, *Rana catesbiana*. Tadpole hemoglobin has a high affinity for oxygen and virtually no loss of affinity on acidification (the Bohr effect); adult hemoglobin has a much lower affinity for oxygen and a large Bohr effect. [From Riggs (12)]

affinity on acidification (that is, no Bohr effect), whereas frog hemoglobin has a very large Bohr effect (Fig. 8).

It is clear therefore that hemoglobin, like the pigment of rod vision, metamorphoses in the bullfrog at the time of anatomical metamorphosis. Both substances are conjugated proteins. In the rod pigment, it is the prosthetic group, retinene, which changes; the protein opsin, so far as known, remains unaltered. In hemoglobin it is the protein, globin, which changes; the prosthetic group, heme, is the same always (13).

Recently Frieden *et al.* (14) have described a third change in the proteins of this species at metamorphosis (Fig. 9). In the bullfrog tadpole the predominant proteins of the blood plasma are globulins. At metamorphosis, the protein concentration of the plasma doubles, and albumins become predominant. These changes can be induced prematurely, just as can anatomical metamorphosis, by administering triiodothyronine.

Still another type of biochemical change has been shown to accompany metamorphosis in frogs and salamanders. Fishes excrete most of their nitrogen as ammonia, whereas land vertebrates excrete their nitrogen primarily as urea or uric acid. Munro (15) showed some years ago that whereas the tadpoles of the frog, *Rana temporaria*, excrete the great bulk of their nitrogen as ammonia, at the metamorphic crisis this animal goes over to excreting its nitrogen primarily as urea (Fig. 10). At this time, also, arginase, the last in the chain of enzymes that forms urea, makes its first appearance in the liver. Recently Munro (16) has demonstrated similar changes accompanying metamorphosis in the toad *Bufo bufo*, the salamanders *Triturus vulgaris* and *T. cristatus*, and the axolotl *Siredon mexicanum*.

These observations make a beginning with the biochemistry of metamorphosis. They show that just as animals in metamorphosis undergo radical alterations in anatomy, so their biochemistry is fundamentally revised at the same time. Indeed both kinds of change, anatomical and biochemical, herald an ecological transition, for they are followed by radical changes of habitat. They mark also an evolutionary transition, for these changes offer the most striking instances we know of recapitulation. The amphibian in metamorphosis seems to repeat in rapid summary the changes which accompanied the emergence of vertebrates from fresh water onto land. The transformation of visual systems and of the

pattern of nitrogen excretion seems to provide clear instances of *biochemical recapitulation*. The changes in hemoglobin also seem to involve aspects of recapitulation (8, p. 337; 17). Whether the changes in serum proteins have this character, it is too early to say. In any case, in metamorphosis the anatomy, the biochemistry, and, shortly afterward, the ecology all are transformed, and frequently in some degree of accord with the animal's evolutionary history.

It is interesting to realize how closely these patterns hold together. An aberration in one of them seems to call forth appropriate aberrations in the others. The mud puppy, *Necturus maculosus*, for example, remains to some degree a permanent larva, never losing its external gills and never emerging from the water. Some years ago I found that adult mud puppies have porphyropsin alone, like a fresh-water fish (8).

The clawed toad, *Xenopus laevis*, a

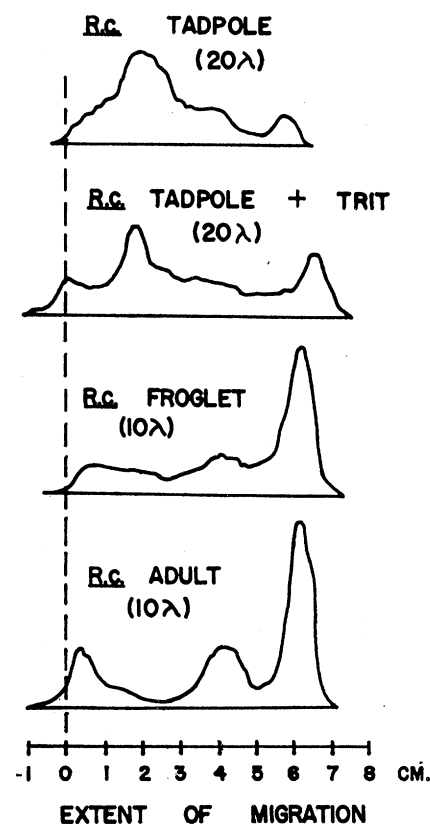


Fig. 9. Biochemical metamorphosis of plasma proteins in the blood of the bullfrog, *Rana catesbiana*. Tadpole plasma contains a predominance of globulins that migrate slowly on electrophoresis at pH 8.6; in the froglet a change to a predominance of rapidly migrating albumin takes place. This change is also induced prematurely by treatment of tadpoles with triiodothyronine (Trit). The volume of serum used is indicated in parentheses. [From Frieden, Herner, Fish, and Lewis (14)]

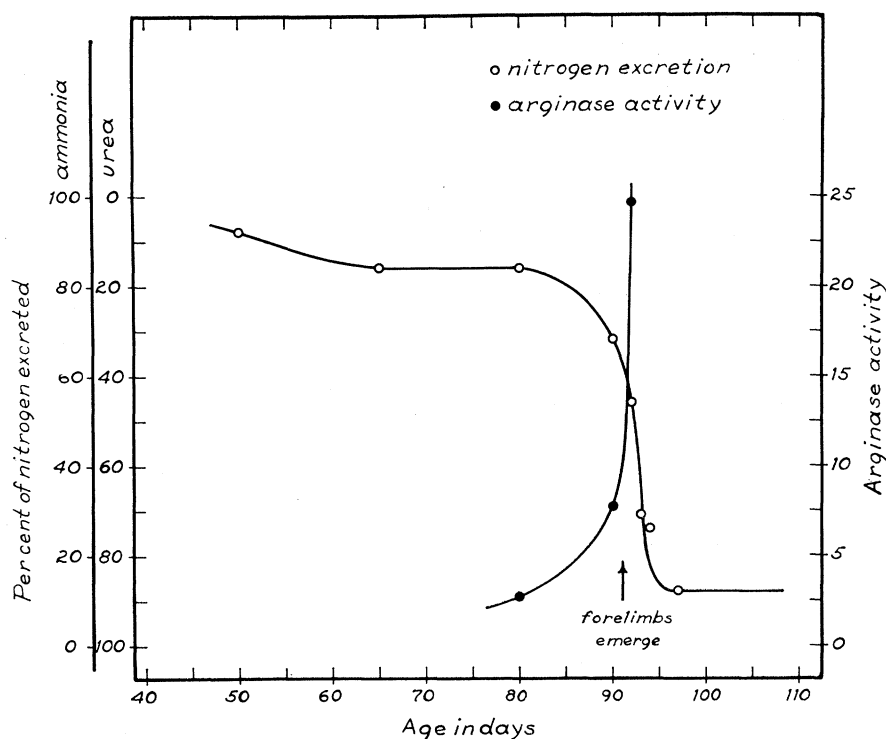


Fig. 10. Biochemical metamorphosis of nitrogen excretion in the European frog, *Rana temporaria*. At the metamorphic crisis, this animal changes from excreting about 90 percent of its nitrogen as ammonia to excreting about 90 percent as urea. Simultaneously, the activity of arginase, the enzyme that hydrolyzes arginine to form urea, rises sharply in the liver. [From Wald (8), drawn from the data of Munro (15)]

member of the peculiar family Aglossa, which possesses neither tongue nor teeth, is a purely aquatic form, which, though it metamorphoses, ordinarily never emerges from the water. Adults of this species possess, in their retinas, vitamin A₂ and porphyropsin almost exclusively (18). Underhay and Baldwin (19) have recently shown that this species also exhibits peculiar changes in the pattern of its nitrogen excretion. As a tadpole it excretes nitrogen primarily as ammonia. At metamorphosis, like other amphibia, it begins to change over toward urea excretion, so that at the height of metamorphosis it excretes a little more nitrogen as urea than as ammonia. Toward the end of metamorphosis, however, it swings back again, so that the adult excretes about three times as much ammonia as urea nitrogen. It is as though this animal, having got ready to leave the water, changed its mind; and both the getting ready and the change of mind are reflected in the nitrogen excretion. Indeed, *Xenopus* can change its mind again; for if kept moist while yet out of water it accumulates huge amounts of urea, perhaps as a device for conserving water such as is practised by the elasmobranch fishes. Its return to water is attended by a massive excretion of urea

accompanied by very little ammonia (20).

We see, therefore, that even the aberrations of amphibian metamorphosis, anatomical and ecological, are paralleled closely by the biochemistry. It is probably true that in all cases in which the anatomy or the ecology changes, the biochemistry also changes. Indeed the biochemistry may have a primary status; the visible alterations in anatomy and ecology may only reflect prior biochemical changes.

Second Metamorphosis

The first requirement of a life cycle is that it be *circular*. Any organism that leaves its natal environment to explore, or grow up in, another must return at maturity to reproduce its kind. The spawning environment is fixed, whatever excursions animals may make as adults, and it is a truism that all animals must return to their natal environment to spawn.

For this reason, any animal that undergoes profound changes preparatory to migrating from its natal environment is likely to undergo a second series of changes in the reverse direction before

returning. Every metamorphosis invites a second metamorphosis.

Let us begin with the common spotted newt mentioned above. This animal begins its life as an olive-green, gilled larva, living wholly in the water. After several months it metamorphoses to a lung-breathing, land-dwelling eft. The color changes to a brilliant orange-red, the skin becomes rough and dry, the lateral-line organs recede (21). The newt now lives 2 to 3 years wholly on land, growing meanwhile almost to full size. Then it undergoes a second metamorphosis: the color returns approximately to that of the larva, and the newt regains the wet, shiny, mucus-covered skin, the keeled tail, the functional lateral-line organs, though not, of course, gills. In this mature state it reenters the water to spawn and live out the remainder of its life.

I have already said that the mature animal possesses porphyropsin, like a fresh-water fish. These animals, however, had already undergone the second metamorphosis. Red efts on examination were found to possess mixtures of rhodopsin and porphyropsin, predominantly rhodopsin (Fig. 11). The second metamorphosis in this species is accompanied therefore by the biochemical metamorphosis of its visual system from a predominantly land type to that characteristic of fresh-water types (8, p. 337).

This, in turn, brings us back to the sea lamprey. This animal has a life cycle

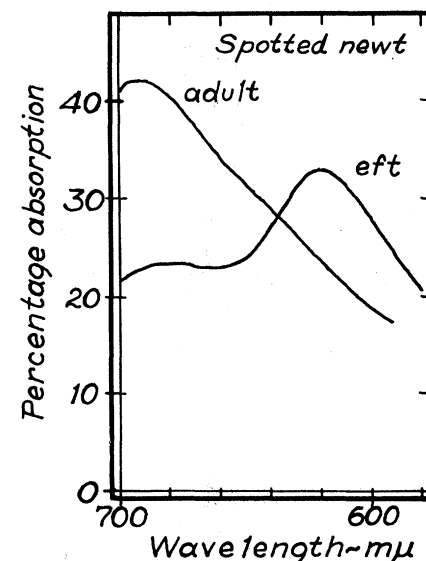


Fig. 11. Second metamorphosis of visual systems in the New England spotted newt, *Diemictylus viridescens*. Retinas of the land-living red eft contain a preponderance of vitamin A₂, with a minor admixture of A₁; retinas of water-phase, sexually mature adults contain vitamin A₂ predominantly or exclusively. [See Wald (8)]

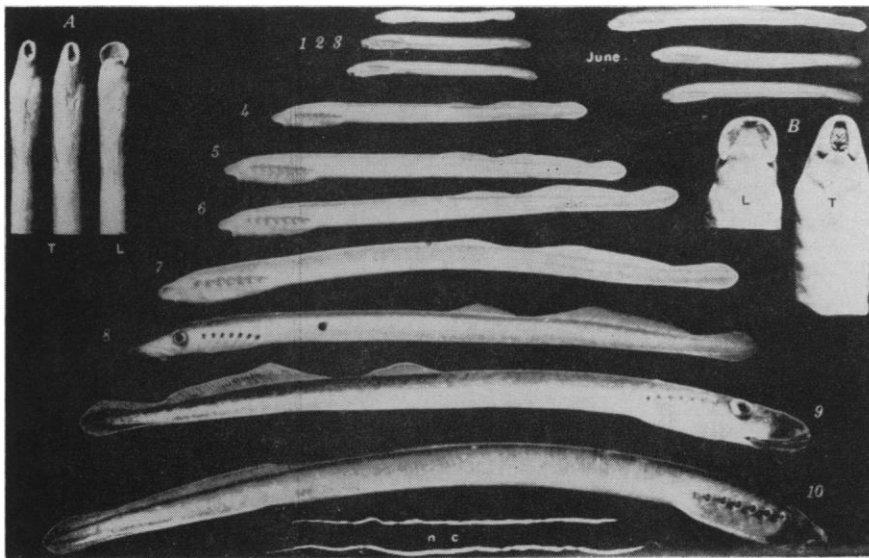


Fig. 12. Development of the sea lamprey, *Petromyzon marinus*. This animal begins its life in streams as a blind larva, buried in mud or sand (stages 1 to 7). Then it undergoes a first metamorphosis while still in this position (stages 8 to 10), preparatory to migrating downstream. Several years later it undergoes a second metamorphosis, to the sexually mature adult, and migrates upstream again to spawn and die. (A, B) Transformation of the mouth at first metamorphosis from the larval, hooded form (L) to the contracted circular form (T). (n c) Notochords of decayed adults found in streams after spawning. [From Gage (22)]

much like that of a salmon (Fig. 12). After passing 4 to 5 years as a blind ammocoete larva, living buried in the sand or mud of its natal stream, it undergoes, while still in that position, a profound metamorphosis, preparatory to migrating downstream to the ocean or a lake for its growth phase. This lasts $1\frac{1}{2}$ to $3\frac{1}{2}$ years. Then the sea lamprey undergoes a second metamorphosis, to the sexually mature adult. The sexes differentiate visibly for the first time: the gonads mature, secondary sex characteristics appear, the males develop a rope-like ridge along the back, and either sex

may assume golden mating tints. Then these animals migrate upstream to spawn (22).

Some years ago I found that the sea lamprey, taken on its spawning migration, has almost exclusively vitamin A_2 in its retina, and I concluded from this that it probably possesses porphyropsin (23). Since lampreys are members of the ancient class Agnatha, which includes the most primitive living vertebrates, I took this observation to support the view that porphyropsin is the ancestral type of visual pigment in vertebrates.

Recently, however, Crescitelli (24) reported that he had extracted rhodopsin from the retinas of this species and pointed out that this goes better with the opposed view, that rhodopsin is the primitive vertebrate pigment.

The specimens of sea lamprey examined by Crescitelli had just metamorphosed from the larval condition and had begun to migrate downstream, whereas the ones I had examined were at the other end of their life cycle, migrating upstream to spawn. On obtaining downstream migrants like Crescitelli's, I confirmed his observations exactly (Fig. 13). The retinas of such animals contain vitamin A and rhodopsin alone. The upstream migrants, however, possess vitamin A_2 and porphyropsin virtually alone (25). We find therefore in this most primitive group of vertebrates another biochemical example of second meta-

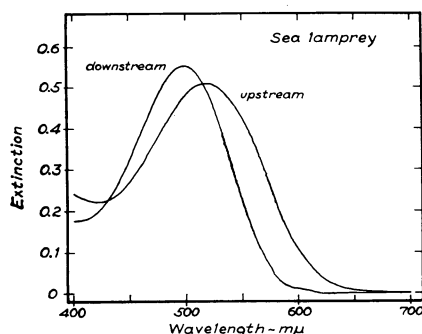


Fig. 13. Second metamorphosis of visual systems in the sea lamprey, *Petromyzon marinus*. The downstream migrants, having recently undergone the first metamorphosis from the larval stage, possess rhodopsin. The sexually mature adults, migrating upstream from the sea to spawn, have porphyropsin alone. [From Wald (25)]

morphosis, like that previously observed in the newt.

Such second metamorphoses expose fundamental characteristics of the metamorphic process:

1) Both the first and second metamorphoses *anticipate* changes in environment. Ordinarily they occur in the old environment and are completed there. They are *preparations* for the new environment, not responses to it.

2) Just as the first metamorphosis prepares the animal to leave its natal environment, so the second metamorphosis prepares it to return, completing the life cycle. It is of the essence of a second metamorphosis to reverse in part the changes which accompanied the first metamorphosis. The two metamorphoses tend to be opposed in direction, anatomically and biochemically.

3) Just as the changes in the first metamorphosis tend to have the character of recapitulations—that is, to coincide somewhat with the animal's evolutionary history—so the changes which occur in a second metamorphosis are likely to be antirecapitulatory, to reverse in direction the sequence of changes that accompanied the animal's evolution.

The last consideration involves a potential source of confusion. As I have already said, a life cycle is circular. If one section of it runs parallel with the course of evolution, another section is likely to run counter to that course. Just as every metamorphosis invites a second metamorphosis, so every associated recapitulation invites a subsequent antirecapitulation. This is only proper, provided it occurs at the point in the animal's history when it is being prepared for the return to the natal environment.

Deep-Sea Fishes; Eels

Heretofore I have discussed only changes in the visual pigments that involved their prosthetic groups. I should like now to discuss another type of change, involving the other component of a visual pigment, the protein opsin.

A short time ago Denton and Warren (26) reported that the visual pigments of deep-sea fishes, instead of having absorption maxima (λ_{max}) near 500 mμ, as do the rhodopsins of surface forms, have λ_{max} near 480 mμ. In consequence, they are orange in color rather than red, and Denton and Warren proposed that they be called chrysopsins, or visual gold. For reasons which appear below, I prefer to call them deep-sea rhodopsins.

This observation has since been confirmed by Munz (27) and by Wald, Brown, and Brown (28). It makes good ecological sense; for the surface light that penetrates most deeply into clear sea water is blue, and made up of wavelengths near 480 m μ , and the rhodopsins of deep-sea fishes are more effective through having their maximal absorption in this region of the spectrum (26).

As might be expected, the transition from surface to deep-sea rhodopsin is not sudden. A preliminary exploration shows that the absorption spectra of the rhodopsins shift more or less systematically with depth from the surface to about 200 fathoms (Fig. 14). We find that throughout such a series the prosthetic group—the retinene—remains the same. It is the opsin which alters (28). We have here a relationship comparable with that familiar in the hemoglobins, all of which possess the same heme joined with a variety of globins, different in every species.

Disregarding the relatively few rhodopsins and porphyropsins which lie in exceptional positions, one sees, therefore, a major transition (from $\lambda_{\max}$ 480 to $\lambda_{\max}$ 500 m μ) in the rhodopsins of marine fishes, correlated with depth, and depending on a systematic change of opsins; this connects with a further major transition from rhodopsin to porphyropsin (from $\lambda_{\max}$ 500 to $\lambda_{\max}$ 522 m μ) correlated with the transfer to fresh water, and depending on the change of chromophore from retinene₁ to retinene₂.

With this we can return to the “fresh-water” eel (*Anguilla*). Carlisle and Denton (29) have recently confirmed our observation that this animal, when taken in fresh water, ordinarily possesses the mixture of rhodopsin and porphyropsin described earlier; but they find that toward the beginning of its spawning migration it goes over to deep-sea rhodopsin (Fig. 15). Whereas the absorption peak of its usual mixture of visual pigments, when the eel is in fresh water, lies at about 505 m μ , that of the animal about to migrate lies close to 485 m μ . Indeed, the rhodopsin of such a “fresh-water” eel preparatory to migration is virtually identical in spectrum with that of the permanently deep-sea conger eel (30).

This is another instance of a second metamorphosis (Fig. 16). The eel, having been spawned in the depths of the Sargasso Sea, journeys as a larva (leptocephalus) to the shores of America or Europe (31). There it metamorphoses to the adult form and usually, though

probably not always, migrates into fresh water for its growth phase. Eventually it metamorphoses again: its color changes, the eyes approximately double in diameter, the digestive system deteriorates. As though getting ready for its return to the Sargasso Sea, it changes also to deep-sea rhodopsin.

My co-workers, Paul and Patricia

Brown, have recently examined such animals at the Stazione Zoologica in Naples (5). The European eel about to migrate seaward seems first to lose its retinal vitamin A₂ and retinene₂ and then to begin to combine vitamin A₁ and retinene₁ with a new, deep-sea opsin. The animal has already progressed far with this, as with the anatomical changes of

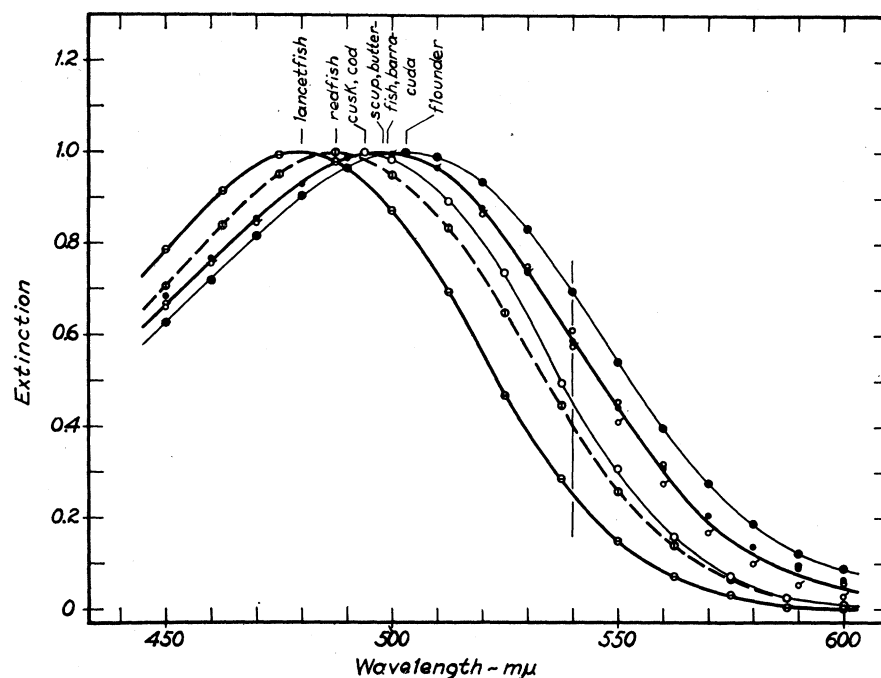


Fig. 14. The rhodopsins of fishes taken at various depths in the sea. That of the lancet fish, found ordinarily below 200 fathoms, has $\lambda_{\max}$ about 480 m μ ; those of surface forms (scup, butterfish, barracuda, flounder) have $\lambda_{\max}$ 498 to 503 m μ . The cusk and cod (from summer depths of 40 to 50 fathoms) have $\lambda_{\max}$ 494 to 496 m μ , and the redfish (from a depth of about 100 fathoms) has $\lambda_{\max}$ 488 m μ . [From Wald, Brown, and Brown (28)]

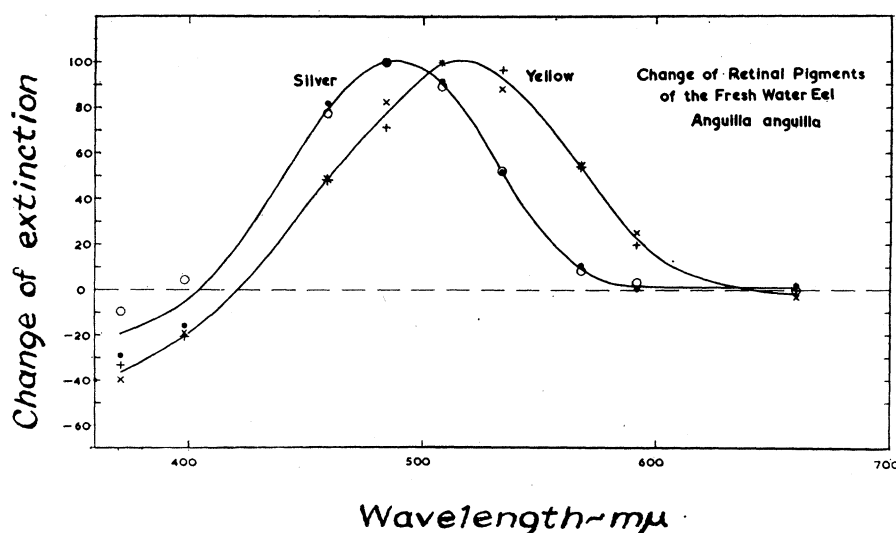


Fig. 15. Second metamorphosis of visual pigments in the European “fresh-water” eel. The yellow eel, prior to second metamorphosis, has a mixture of rhodopsin and porphyropsin (net $\lambda_{\max}$ 500 to 505 m μ) corresponding to the mixture of vitamins A shown in Fig. 4. The sexually mature silver eel, about to begin its spawning migration to the Sargasso Sea, has changed over to deep-sea rhodopsin ($\lambda_{\max}$ about 487 m μ) [Carlisle and Denton (29, 38)].

the second metamorphosis, while still in fresh water.

Such observations can tell us something concerning the larval condition. To my knowledge, no one has yet examined the visual pigment of the leptocephalus larva, but the foregoing discussion suggests strongly that the pigment is deep-sea rhodopsin. Similarly, though no one seems as yet to have examined the retinal pigment of the larval New England newt, our observation that the adult at maturity metamorphoses to porphyropsin implies that this is also the larval pigment. Again, since the blind ammocoete larva of the sea lamprey metamorphoses to an eyed adult possessing rhodopsin, this is the first visual pigment to appear in this

species. Yet the fact that in the second metamorphosis the pigment changes to porphyropsin implies that the latter represents the true, albeit missing, larval type. That is, since the second metamorphosis involves some measure of return to the larval condition, it can tell us something of the larval state, even of larval properties which have been lost in the course of evolution.

Land Vertebrates

Land vertebrates still pursue their embryogeny in water, but they have brought the water ashore. In a sense they are erstwhile amphibia which have carried wa-

ter ashore in which their embryos go through the larval stages and first metamorphosis. They have developed two special devices for this: the boxed-in or cleidoic egg, and viviparity. Amphibia still experiment with both. Certain of them—for example, the American red-backed, slimy, and worm salamanders—lay eggs on land within which the larvae complete their entire development. Others—such as the European black salamander, *Salamandra atra*—retain the eggs in the body until the young are fully formed. The European spotted salamander, *S. maculosa*, ordinarily lays its eggs in streams, but if it cannot reach water, permits them to develop internally.

One might hope, therefore, to find residues of metamorphosis in the embryogeny of land vertebrates, and in this one is not disappointed. Anatomical residues abound; they were the original source of the idea of recapitulation and were principally responsible for its early overexuberance. The embryo of a land vertebrate undergoes an anatomical metamorphosis approaching that of an amphibian. Unlike a larval amphibian, it never has functional gills; but, for a time, it does of course have gill slits, as well as other evidences of earlier aquatic life.

One finds biochemical metamorphosis also in the embryos of land vertebrates, and it includes some of the same changes with which metamorphosis in amphibia has already made us familiar.

So, for example, Joseph Needham (32) has shown that the chick embryo developing in the egg displays a changing pattern of nitrogen excretion (Fig. 17). It begins by excreting about 90 percent of its nitrogen as ammonia, like a fish; then changes to excreting up to 90 percent as urea, like most adult amphibia; and in the last stages of incubation excretes about 90 percent as uric acid, as do adult birds.

In general, vertebrates hold the osmotic pressures of their body fluids within narrow limits. In the various groups of vertebrates the blood osmotic pressure takes characteristic values, correlated to a degree with the ecology, and perhaps also with the phylogeny (see 8, p. 337). Thus, the freezing-point depression (Δ F.P.) of the plasma in freshwater fishes and amphibia lies at 0.45° to 0.55° C, whereas adult birds and mammals exhibit values of 0.55° to 0.65° C. Recent measurements in the developing chick (33) show that the fluids of the early embryo have freezing-point depressions at about 0.47° C (Fig. 18). The fluid osmotic pressure rises throughout development, with a final spurt at hatch-

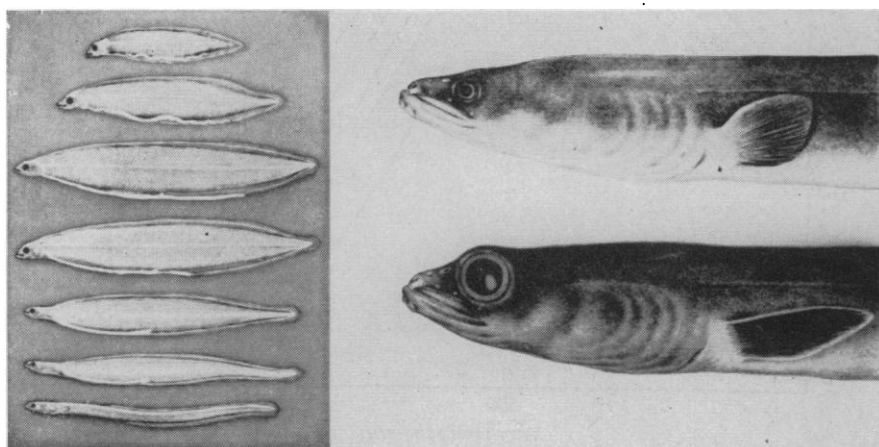


Fig. 16. Development of the American eel, *Anguilla rostrata*. (Left, top to bottom) Three larvae, or leptocephali, of various sizes; then three larvae undergoing the first metamorphosis; and the metamorphosed "glass eel." The uppermost three pictures are enlarged 2.7 times relative to the others. (Right) The second metamorphosis: (top) American eel in the "green" stage; (bottom) a European eel in the "silver" stage, ready for its spawning migration. Note particularly in the latter the approximate doubling in diameter of the eye. This large-eyed stage has not yet been observed in the American species. [From Vladykov (37)]

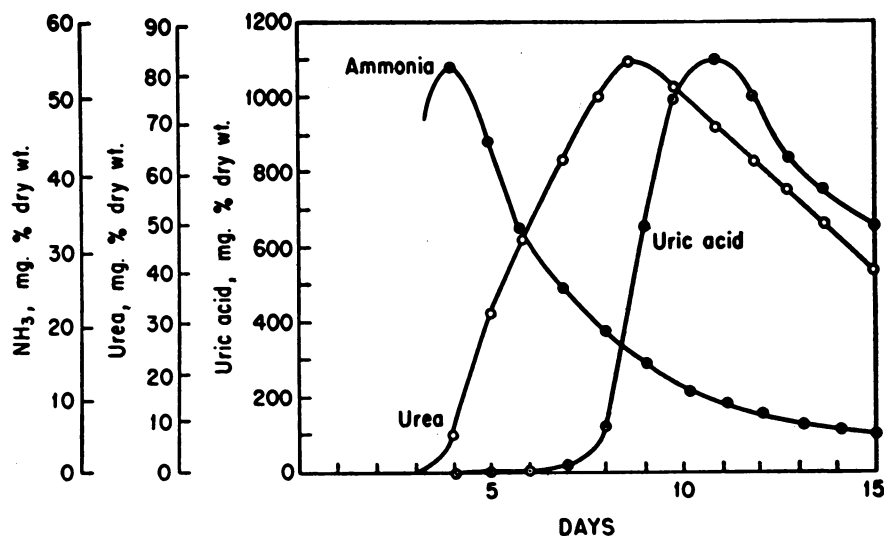


Fig. 17. Biochemical metamorphosis of nitrogen excretion in the developing chick. During incubation in the egg, the chick embryo successively excretes about 90 percent of its nitrogen as ammonia, then as urea, and finally as uric acid. [After Needham (32)]

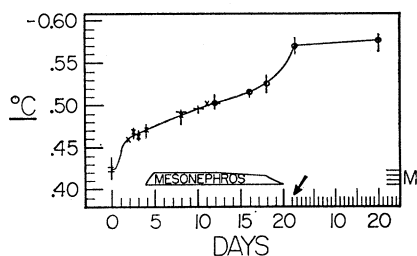


Fig. 18. Biochemical metamorphosis of fluid osmotic pressure in the developing chick. (Ordinates) Freezing-point depression, a measure of osmotic pressure; (abscissae) days of incubation, to hatching on the 21st day (arrow), and days thereafter; (=) unincubated egg white, (x) sub-germinal fluids, (-) amniotic fluids, (o) bloods. The embryo begins with osmotic pressures characteristic of fresh-water fishes and amphibia and ends with the much higher osmotic pressure characteristic of mature birds and mammals. The duration of functional activity of the mesonephros (M) is also indicated. [From Howard (33)]

ing that brings it to the adult value (Δ F.P. = 0.58°C). That is, the embryo begins with a fluid osmotic pressure characteristic of fresh-water fishes and amphibia and ends with that characteristic of mature birds (34).

Again, Hall (35) has shown that during the embryonic development of the chick its hemoglobin changes radically, continuously losing affinity for oxygen, so that in an adult chicken more than twice the oxygen pressure is needed for half-saturation than is needed in a ten-day-old chick (Fig. 19). These changes persist for some time after hatching. They are similar in direction to the change in hemoglobin that accompanies metamorphosis in the bullfrog.

Comparable changes in hemoglobin accompany the embryonic development of all mammals so far examined. It is now well recognized that in mammals generally, man included, fetal hemoglobin is a different species of molecule from maternal or adult hemoglobin (see 36) (Fig. 20). Always—with the possible exception of man—the change in oxygen affinity is in the same direction, a loss of affinity as development progresses. The fetal and adult hemoglobins of mammals differ also in many other ways: in electrophoretic mobility, sedimentation rate, resistance to alkali, immunological specificity, solubility, crystal shape, and amino acid composition (see 8, references). All these changes involve the

globin moiety of hemoglobin; the heme is the same always.

The phenomenon of metamorphosis, biochemical as well as anatomical, extends therefore beyond the amphibia and fishes to include the land vertebrates, both egg-laying and placental.

Do land vertebrates exhibit also vestiges of a second metamorphosis? I suppose that puberty is so to be regarded. To be sure, this does not prepare a land vertebrate to migrate, for the natal environment is now segregated, and puberty prepares the animal only to mate. Here only one representative cell—the spermatozoon—completes the return to the natal environment; and this, of course, undergoes a profound metamorphosis before being launched upon a migration as formidable, relative to its size, as that of any salmon.

Conclusion

Metamorphosis is a basic and general phenomenon, common to the whole vertebrate stock. It includes anatomical, physiological, and—perhaps prior to these—biochemical components, all de-

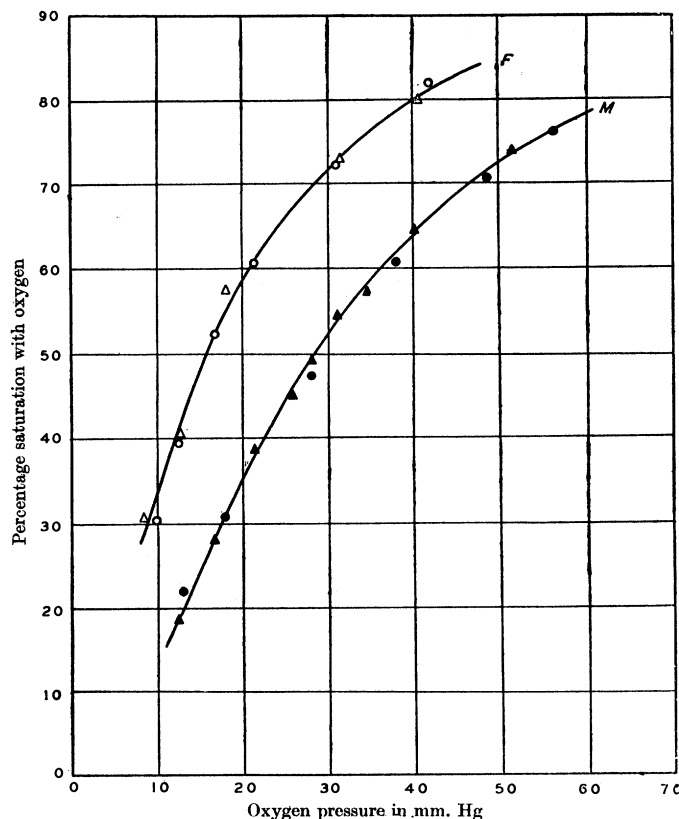
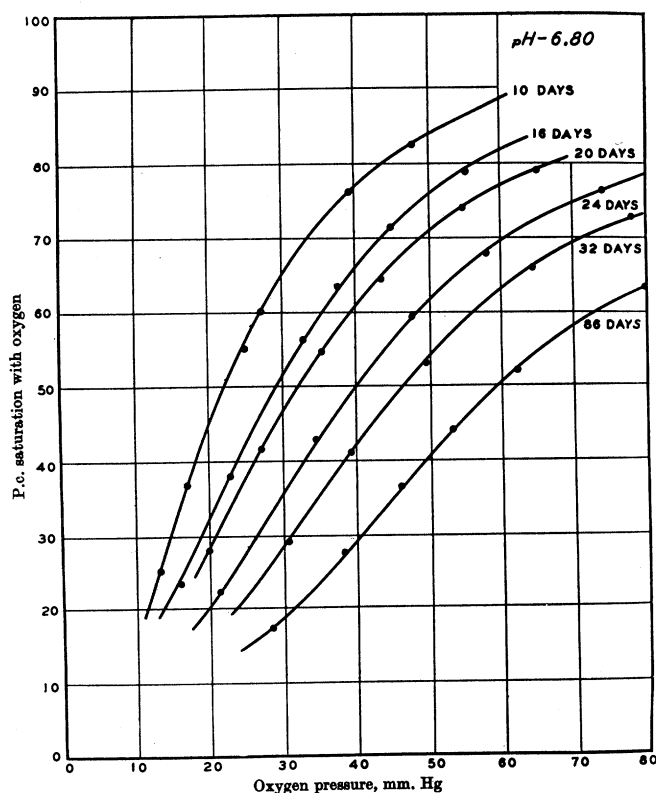


Fig. 19 (left). Biochemical metamorphosis of hemoglobin during development of the chick. Measurements on dilute solutions of hemoglobin buffered at pH 6.80 and equilibrated with oxygen at 37°C . The affinity for oxygen decreases regularly from the tenth day of incubation, and this change continues for some time after hatching. [From Hall (35)]. Fig. 20 (right). Biochemical metamorphosis of hemoglobin in a placental mammal. Oxygen equilibrium curves of hemoglobin from a goat fetus (F) and from the mother (M). The fetal hemoglobin has almost twice as high an affinity for oxygen as the maternal, measured in solution at 37°C and pH 6.8. Both hemoglobins were obtained at 15 to 18 weeks' gestation. [From Hall (36)]

signed to prepare the animal to leave its natal environment. Necessarily, in order to reproduce, the animal must eventually return, so completing its life cycle; and its return may be prepared for by a second metamorphosis, in some aspects the reverse of the first.

Our history as vertebrates is not dust to dust but water to water. From this point of view Nicodemus' great question can be given a broad and positive biological answer. Every animal can and must return to the "womb"—not, indeed, to be born again, but to bear the next generation. For a catadromous fish, the "womb" is the sea; for anadromous fishes and amphibia, a pond or stream; for land vertebrates, a uterus or egg. The question raises additional problems only for man, and then only when the sense of return is toward the womb of the mother rather than that of the mate.

References and Notes

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38. For this figure, showing as yet unpublished measurements of Carlisle and Denton (29), I am very grateful to Dr. E. J. Denton.

Hazard to Man of Carbon-14

What problems are encountered in the quantitative estimation of the biological hazards of carbon-14?

John R. Totter, M. R. Zelle, H. Hollister

Carbon-14 is an isotope of the chemical element carbon. As such, it forms the same chemical compounds and, as part of the organic molecules built around the carbon atom, becomes part of living tissue. Carbon-14, though vir-

tually indistinguishable chemically from the other isotopes of carbon, is radioactive, emitting low-energy beta particles (0.05 Mev average) and having a half-life of about 5600 years. Therefore, carbon-14 is a potential hazard to man, for

in his body it is emitting radiation that can affect living cells and, additionally, is itself undergoing change—transmutation—by decay to another element, nitrogen, of different chemical characteristics.

This article considers briefly some of the problems encountered in the quantitative estimation of the biological hazards to man of carbon-14.

Possible Mutagenic Effect of Carbon-14 Transmutation

Most discussions of the genetic effects of radioactive isotopes present in the body have considered only the effects of the radiation emitted during radioactive decay. However, the transmutation of radioactive atoms which have been