VIGNETTE

Ross Granville Harrison (1870–1959) and Perspectives on Regeneration

JOURNAL OF EXPERIMENTAL ZOOLOGY
PART B
MOLECULAR AND DEVELOPMENTAL EVOLUTION

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ABSTRACT

Historical case studies can serve as cautionary tales, reminding us to reflect on underlying assumptions and on limitations of any particular approach. Ross Harrison's work recorded at the beginning and end of his career in the *Journal of Experimental Zoology* reveal his own morphological and experimental convictions, as they played out in his studies of regeneration. A closer look at this particular example of Harrison's contributions offers a perspective from which to view current studies of regenerative phenomena and assumptions about appropriate research approaches and the driving questions involved. *J. Exp. Zool. (Mol. Dev. Evol.) 314B:607–615, 2010.*

J. Exp. Zool. (Mol. Dev. Evol.) 314B:607–615, 2010

How to cite this article: Maienschein J. 2010. Ross Granville Harrison (1870–1959) and perspectives on regeneration. J. Exp. Zool. (Mol. Dev. Evol.) 314B:607–615.

REGENERATION

Regenerative medicine is often imagined, by the supportive public, as the literal process of regenerating and thereby replacing tissues or cells that are not working properly. Yet the field as a whole actually includes two rather different approaches. One involves the attempt to directly replace lost cells or tissues that will allow restoration of lost function, whereas the other involves developing new cells or tissues that may be different from the original to compensate for lost structure and function in new ways that may be different from the original. As the Department of Health and Human Services put it in 2006 in setting up programs in regenerative medicine: "This new field holds the realistic promise of regenerating damaged tissues and organs in vivo (in the living body) through reparative techniques that stimulate previously irreparable organs into healing themselves. Regenerative medicine also empowers scientists to grow tissues and organs in vitro (in the laboratory) and safely implant them when the body is unable to be prompted into healing itself" (Health and Human Service, 2006).

The understanding of such differences has a long history, and our current approaches have roots dating back over a century. Most notably, attempts at the very beginning of the 20th century to understand the different phenomena of regeneration led Thomas Hunt Morgan to summarize contemporary studies in a series of lectures delivered at Columbia University and presented in his synthetic volume *Regeneration* in 1901 (Morgan, '01; Maienschein, '91a,b; Sunderland, 2008). There Morgan noted that researchers had observed diverse regenerative phenomena for several centuries, including especially with hydra, earthworms, and planarians. The question remained how regeneration occurs. Is it that existing tissue is stimulated to add new tissue that replaces the structure and function of the damaged part (which Morgan called "epimorphosis")? Or is existing tissue modified so as to take on the missing structure and function (which he called "morphallaxis"). He then continued to distinguish "homomorphosis" in which the resulting part is like the original, and "heteromorphosis" where the result is different in structure and/or function. Third, he noted that some regenerative processes yield renewed function but not structure, or the other way around (Morgan, '01; especially p 23–25).

Morgan's book laid out the existing empirical and experimental evidence related to regeneration, and a discussion of existing theories of the cause. He rejected evolutionary explanations and instead focused on factors internal and external to the organism in question, looking in particular at whether "organization" could offer explanatory force. After laying out the range of existing questions and suggestions for understanding regeneration, he largely moved away from the field and turned to other studies of embryology and then genetics (Allen, '78, discusses Morgan's evolving research program). Instead, his valuable summary defined the problem and laid the foundation for others,

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Received 9 August 2009; Revised 3 April 2010; Accepted 28 May 2010
Published online 17 August 2010 in Wiley Online Library (wileyonlinelibrary.com). DOI: 10.1002/jez.b.21368

including his fellow graduate student from Johns Hopkins and friend Ross Granville Harrison.

This article addresses aspects of Harrison's regeneration studies. First, what did Harrison conclude about the nature and causes of regeneration at the beginning and end of his long productive career? Second, what underlying epistemological assumptions did he make about how to carry out the study of such complex internal processes and what difference did they make? In conclusion, what can we learn from this historical example?

ROSS GRANVILLE HARRISON ON GROWTH AND REGENERATION

Harrison served as founding managing editor of the *Journal of Experimental Zoology* from 1903 through volume 105 in 1946, with Morgan as a central member of the editorial board (Harrison, '45). Along with Hans Spemann, Harrison was considered the leading experimental embryologist focused on fundamental questions of understanding development of his time. It is worth noting that two of Harrison's most significant articles addressing regeneration and what he saw as the related phenomenon of wound repair were published in the *JEZ*. His earlier work set the stage for these major papers.

Harrison received his Ph.D. from the Johns Hopkins University with a study of symmetry and limb development. His dissertation advisor William Keith Brooks encouraged Harrison to continue his studies in Germany, where he received an M.D. for his work with Rudolf Leuckart that extended his work on teleost limb development (see Nicholas, '61a,b for biography). Harrison's first professional position was in the medical school at Johns Hopkins, where he took up methods of heteroplastic grafting in frogs, inspired by the work of Gustav Born and Eduard Pflüger. An article in 1898 looked at "The Growth and Regeneration of the Tail of the Frog Larva," for example, and began with Born's methods for grafting pieces of tail from different species (Harrison, 1898).

Harrison noted that after various methods for cutting and wounding the tadpoles, "The regularity with which the tail is regenerated is remarkable although, even in outward appearance, the regenerated appendage never becomes exactly like the original" (Harrison, 1898; p 445). He referred to his plates, which included attempts to photograph living tadpoles as well as photographs based on preserved specimens. This work gives one of the first indications of his epistemological convictions, as it is clear that Harrison thought it better if he could provide photographs of live specimens. Yet he found it very difficult to capture clear and useful pictures (Harrison, 1898; p 434). His discussion of the results also showed that he saw normal growth, grafting results, wound repair, and regeneration as all closely related phenomena with the same underlying causes, all depending on the growth and movement of cells within structures.

The place of cells mattered. Therefore, "The question then arises, whether a tail will be produced under the influence of the position of the regenerating center with regard to the whole organism, or whether the elements of the transplanted stump retain their original orientation and strive to reproduce the lost body. By varying the position of the transplanted piece as mentioned above, it is possible to test the influence of functional activity upon the regenerating parts"(Harrison, 1898; p 449). Here Harrison referred specifically to Morgan's distinction of epimorphosis and morphallaxis, noting that it was difficult to determine the causes and forces in the regenerative process but that doing so was a fundamental question about development. How much is self-differentiation, responding to the needs and constraints of the organized organism, and how much is opportunistic response to the changed external conditions: that was a fundamental question underlying development generally.

Harrison became fascinated by this set of core questions. He raised the question whether it might be possible to discover more about the capacities of cells and tissues to differentiate, under what conditions, by carrying out the ultimate transplantation experiment. Here, he would not just transplant pieces of tadpole tail from one place to another, but he would take cells out of the organism altogether and into an artificial culture medium. This work, reported in 1907, led Harrison to carry out the first ever successful tissue culture and also the first stem cell experiment as the cells he transplanted were undifferentiated neuroblast cells (Harrison, '07; Maienschein, '83; Witkowski, '85).

Harrison's tissue culture techniques attracted immediate attention, and Alexis Carrel and others took up the methods for medical purposes that Harrison did not choose to pursue himself (Stapleton, 2004; Landecker, 2007). Harrison was perhaps disappointed that his experiments did not immediately and definitively settle the question he had been addressing, namely how nerve fibers develop. That is a long story that has been told elsewhere so we need not repeat it here (Maienschein, '83, '91a,b).

What is important for our purposes is the research directions after Harrison left Johns Hopkins in 1907 for Yale University in 1907, where he was appointed both Chairman of the Department of Zoology in Yale College and also Bronson Professor of Comparative Anatomy in the Medical School. This was the first such appointment, and the archival records suggest that the administration hoped for Harrison to help modernize the rather traditional natural historical work in Zoology while also connecting developmental research with medical applications. Harrison intensely disliked teaching introductory undergraduate courses, and was much happier training graduate students and medical school students (Nicholas, '61a,b; p 140). Even though he often published in medical journals and discussed the possible uses of the work done by himself and others, he remained a committed experimental embryologist rather than a clinical researcher.

At Yale, Harrison saw the need to present his evidence about nerve fiber development in different ways that would be more convincing. He laid out the case in a compelling article in the journal he edited, the *JEZ*, in 1910. This long article carried his

tissue culture research forward in ways that both show his interpretations of differentiation and regeneration, and also his epistemological convictions. Again, this work has been discussed in considerable detail elsewhere and can be summarized briefly here (Maienschein, '83, 2003; Witkowski, '85). Harrison raised the question whether nerve cells become differentiated as such, with fiber growth, because of protoplasmic movement or because of differentiation without movement (Harrison, '10; p 789). He transplanted pieces of tissue with neuroblast cells known to give rise to nerve fibers into a hanging drop of frog lymph and found that "The fibers bear a perfect morphological resemblance to undoubted nerve fibers found in sections of normal embryos of a corresponding stage of development" (Harrison, '10; p 791). Therefore, because of the structural similarities, he concluded that the experimental processes were identical to normal processes. Experimental manipulations are not only legitimate but actually preferable to studying the living organism itself, Harrison concluded. The method "renders it possible to keep [the cells and tissues] under direct continuous observation, so that all such developmental processes as involve movement and change of form may be seen directly instead of having to be inferred from series of preserved specimens taken at different stages" (Harrison, '10; p 792).

To those who complained that such experiments could not reflect normal conditions, Harrison responded that "There are many who would deny to this type of experiment validity in elucidating the phenomena of normal development, maintaining that the experimental conditions are too radically different from the normal to be of value in interpreting the latter." (Harrison, '12; p 183). They have a point, he conceded, yet why do we adhere so tightly to our idea of the normal as "the object as it occurs in nature, the organism as a whole, which to many seems to be a sort of fetish not to be touched lest it show its displeasure by leading the offender astray? There is no real ground for maintaining this attitude" (Harrison, '12; p 184).

Instead, researchers need to take up further experimentation, to take living systems apart to look inside and analyze processes. What we need, Harrison urged in 1912, is new techniques, new ways of analyzing, and more experimental results that lead to understanding of embryonic development and differentiation. His message and his reasoning obviously resonate today, as we gain the ability to take apart embryos and rebuild them with constructed combinations of cells and gene activations, developmental pathways, and other decidedly not natural interventions.

TRANSPLANTATION AND REGENERATION

Harrison's work with the National Research Council surrounding both World Wars and in other administrative capacities gave this leading developmental biologist a sharpened awareness of the potential clinical applications of studies on development and regeneration. Though he did not take his research from the bench to bedside himself, he respected the value of clinical research and encouraged others to do so (Harrison, '44).

Continuing into the 10s and 1920s, Harrison was interested in extending those transplantation studies that had led to his stem cell work. In this line of research, Harrison, Hans Spemann, and others carried out a number of descriptive "what if" experiments. What if I cut out this bit here, what will happen? Will a lens still develop if the eye vesicle is removed, Spemann asked, for example. Or what if I cut off this bit here and move it to a new host site: will it develop the way the donor bit would have normally or as the host site would have? There are excellent explorations of the rich history of such transplantation studies, which led to what Harrison frequently called embryology's "gold rush" of the 1930s and to ideas of induction and the organizer led by Spemann (Hamburger, '88).

What is important overall about this period of traditional experimental embryology is its emphasis on looking closely at the contribution of each physical part of the embryo. Asking "what if" something changed was a way to get at relationships over time. How did the presumptive eye vesicle relate to the eye lens that emerged later, for example? To what extent was a part already determined by its place in the normal organism and to what extent could its role be regulated and changed by the environment? What role does the internal environment of the organism and the way different parts relate to each other play in their differentiation? If there were "inducers" or even "organizers" as Spemann suggested, what were they and how did they work? These are driving questions of experimental embryology. And they were addressed by a wide range of heteroplastic transplantation and related experiments, which raised fundamental questions about how organisms respond to the interventions (Harrison, '34).

Cutting, transplanting, observing, and reorganizing methods of experimental embryology proved highly productive (for example, see Gilbert, '91, for more discussion). Historians have studied this work, but there is room for much closer analysis, especially of the transplantation studies that at the time did not seem to have "paid off." For our purposes here, however, I jump ahead to Harrison's last publication, also in the *JEZ* and following the same lines of research, and return to issues of regeneration in the context of wound repair.

ROSS HARRISON ON WOUND REPAIR AND REGENERATION

In 1947, Harrison had just stepped down as editor of the *JEZ*, and he had retired from Yale in 1938 as a result of the university's mandatory retirement policy. He served as Chairman for the National Research Council from 1938 during the critical war years until 1946. In that role, he worked closely with Frank B. Jewett, who served as president of the National Academy of Sciences. They worked on such things as policies for rationing and increasing supplies of antibiotics including penicillin, and

policies related to educating medical professionals for military service (Nicholas, '61a,b).

Also in 1938, Harrison joined the Science Committee of the National Resources Planning Board, and he chaired President Roosevelt's Committee on Civil Service Improvement in 1939. As Harrison's biographer and former student John S. Nicholas noted, "In these capacities he created a liaison between governmental agencies [in] which he was not hesitant about advising on the use of scientists in areas where they could do the greatest service. He was alert to the necessity for change in the Divisions of the Research Council and carried these forward in a quiet and deliberative way." When praised for his work, Harrison characteristically played down his own role and replied "It is easy to work effectively when one finds such cordial response and such willingness to sink minor differences of opinion for the common good" (Nicholas, '61a,b; p 150–153).

This period focused on public service and spent in formal retirement seems to have allowed Harrison to step back and reflect on the body of work he had been pursuing for so long. It also allowed him to decide what problems he wished to take up again. He acknowledged receiving funding from the Rockefeller Foundation, the Carnegie Corporation, and the Carnegie Institution of Washington for new work that made up his last major publication. His paper on "Wound Healing and Reconstitution of the Central Nervous System of the Amphibian Embryo after Removal of Parts of the Neural Plate," appeared in 1947 with Nicholas serving as the *JEZ*'s second managing editor and Harrison serving on the board (Harrison, '47).

In this article, Harrison returned to the topic where he had begun his study of regeneration, looking originally at frog differentiation and development and asking about the experimental conditions under which the embryo recovers its structure and presumably function. Turning in this work from frogs to the salamanders that he had come to know well through his studies of normal developmental stages, Harrison reviewed literature on the subject, added his own recent observations, and prepared and presented his data in noteworthy ways that reflect his underlying epistemological assumptions about what it takes to persuade his readers.

It is worth revisiting this contribution to the history of studies of regenerative medicine. Or I should say that it is worth visiting this article in the first place for most of us, as active researchers rarely go back this far without provocation and it is worth reflecting on what is the same or different from current studies of regenerative medicine.

Harrison had been invited to present the Silliman Lectures at Yale, as Spemann had done (addressing similar topics) in 1933 and he spent much of 1946–1949 preparing (Spemann, '38; Nicholas, '61a,b; p 153). Harrison disliked presenting public lectures, so this was a challenge, and he found it impossible to meet the expectation that he would write up the lectures as a book. Instead, his long-time faithful assistant Sally Wilens edited a volume of his papers related to his topic after his death (Harrison, '69). Instead of a grand summary book, then, Harrison's own last major publication related to wound healing and the nervous system.

He began where he had been nearly a half century earlier with his studies of the nervous system. The central nervous system, Harrison began his 1947 article, seemed to have "considerable powers" to heal and reconstitute the normal even in the face of experimental intervention and damage (Harrison, '47; p 47). For this review, he started with the neurula developmental stages. It was very important that Harrison had worked out the normal stages of salamander development and had established the accepted standard, so he knew precisely what should happen under normal conditions and could compare experimental cases to the relevant normal stage (Harrison, '69 for published normal stages that had been circulated to his students for decades).

Asking what differences result because of particular experimental interventions was a logical step. As Harrison found under some circumstances, the neurula was able to respond by adjusting to abnormal circumstances in what looked just like normal ways, though it might take a little while to do so. Or as Harrison presented it in 1947, the neurula and presumably the central nervous system had a powerful capacity to reconstitute the normal and to correct defects caused experimentally in the neural plate. Harrison found particularly intriguing the way the central nervous system responds to damage and the powers of wound healing that persist into late larval and even adult life. He noted similarities of healing processes in the neural plate with wound healing after damage in the remainder of the ectoderm, and the accompanying increased cell division activity.

As Harrison explained, "The incentive to the present studies came from a series of experiments originally designed for investigating the development of the ear, in the course of which certain interesting observations were made on the closure of the neural tube after removal of one lateral half of the neural plate in the region of prospective hindbrain." Material clearly moved from the uninjured side to the defective side, with hyperplasia in the form of increased mitotic activity. This led to the observation that "The kinetics of this is also not understood and questions are raised, which, even if they cannot be answered at present can at least be formulated" (Harrison, '47; p 28–29).

Part of formulating the question involved pulling together the diverse lines of experimentation and comparing the competing interpretations. Some researchers felt that healing occurred as the rest of the embryo extended its activity to heal the wound, whereas others concluded that the damaged part was repairing itself through generation of new cells, just as Morgan had suggested long before. Questions also remained from other studies about the extent to which the healing was superficial or more extensive, in particular whether the neural tube was restored completely or only partially.

The most active researcher in the field was Harrison's former Ph.D. student Samuel Detwiler (who received his Ph.D. in 1918, named his second son Ross Harrison Detwiler, and whose research followed that of Harrison and Spemann with a focus on neural developmental) (Detwiler, '36, '45; Nicholas, '61a,b). As Harrison and Detwiler remained in active regular contact, with Detwiler sending articles to the *JEZ* and Harrison discussing results with him, it is not surprising that Harrison would take up this study of regeneration after a series of publications by Detwiler on experiments with developing brain and their results.

Harrison carried out a sequence of experimental manipulations with clearly focused questions and results getting closer to more subtle questions and preliminary answers. His opening illustration showed his experimental methods. Using what had by then become his favorite research subject *Amblystoma punctatum* (later renamed *Ambystoma*), he carried out a set of experiments that would take progressively more extensive excisions from the neural plate. His Figure 1 illustrated the different cuts made on different specimens. His goal in carrying out successive cuts was to determine what difference it made to take tissue from only one side of the median line vs. from both sides, and what difference if the cut were more or less extensive. Does the embryo compensate by producing new materials to add at the cut edges, or to what extent do cells migrate or become transformed from other places to fill the gap? In particular, can cells migrate across the median and adapt to new circumstances? He summarized the resulting effects on the embryo's exterior in photographs presented in Figure 2 (his Figs. 2–14). But this does not show what has happened inside, which requires the photographs of cross-sections that he also presented (Fig. 3 his Plate). These illustrate clearly how cells reach across and move into new territory, becoming differentiated in new ways, to respond to the changing environmental conditions caused by the wound.

The organization of this 56 page article shows Harrison's underlying assumptions in the context of contemporary understanding of differentiation in wound repair and regeneration. I summarize the different experimental manipulations and reported results, with Harrison's section title in italics and my comments in parentheses:

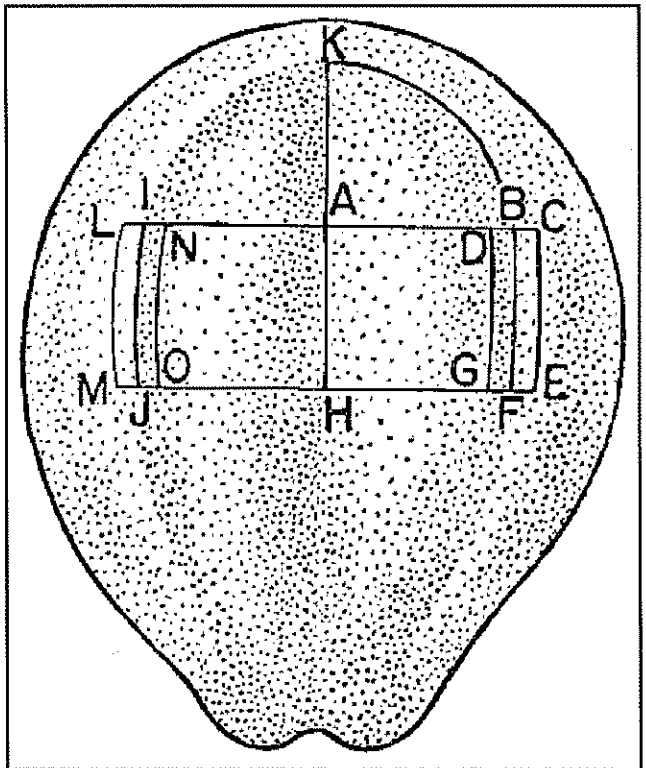

Figure 1. Dorsal view of embyo *A. punctatum*, stage 14, to show the different operations. ABFH, ordinary unilateral excision cut laterally on crest; IBFJ, bilateral operation cut on crest; NDGO, bilateral operation cut inside crest; LCEM, bilateral operation cut outside crest; KBFH, operation for removal of entire half of presumptive brain. × 12.5.

- *Closure of the single neural fold after removal of one lateral half of the prospective hindbrain* (usually removed in the early neural plate, around stage 14, in *A. puntatum* (*maculatum*). The neural tube heals roughly the same in each of the following cases, but other details vary as follows).
  - *Wound left uncovered, mesoderm intact* (observations and serial cross sections show an almost completely healed wound, though the process of closing the neural tube is slow).
  - *Wound covered by ectoderm, mesoderm intact* (the addition of an ectodermal graft over the wound slows the process a bit; the cells do not fuse with the neural crest layer at first and produce an uneven appearance).
  - *Mesoderm removed, wound left uncovered* (the increased damage leads to slower recovery and more frequent failures to heal because of some mechanical difficulties as the ectoderm moves over endoderm without mesodermal mediation. Neural crest seems to be distributed abnormally, which likely has an effect as well).
  - *Mesoderm removed, wound covered with ectoderm* (no different from the previous cases).
- *The effect of the operations on the visceral skeleton* (as the visceral skeleton derives from neural crest, damage to the neural crest predicts likely damage to the skeleton, especially when the mesoderm is damaged. This does occur, though some cases develop normally. Results "merely show that the mesoderm has a part in guiding the movements of the neural crest, which are both extensive and complicated" (Harrison, '47; p 42)).
- *The mode of closure of the neural tube and its effects upon the form of the hindbrain* (two different results from the same experiment suggest that very small changes can lead to a

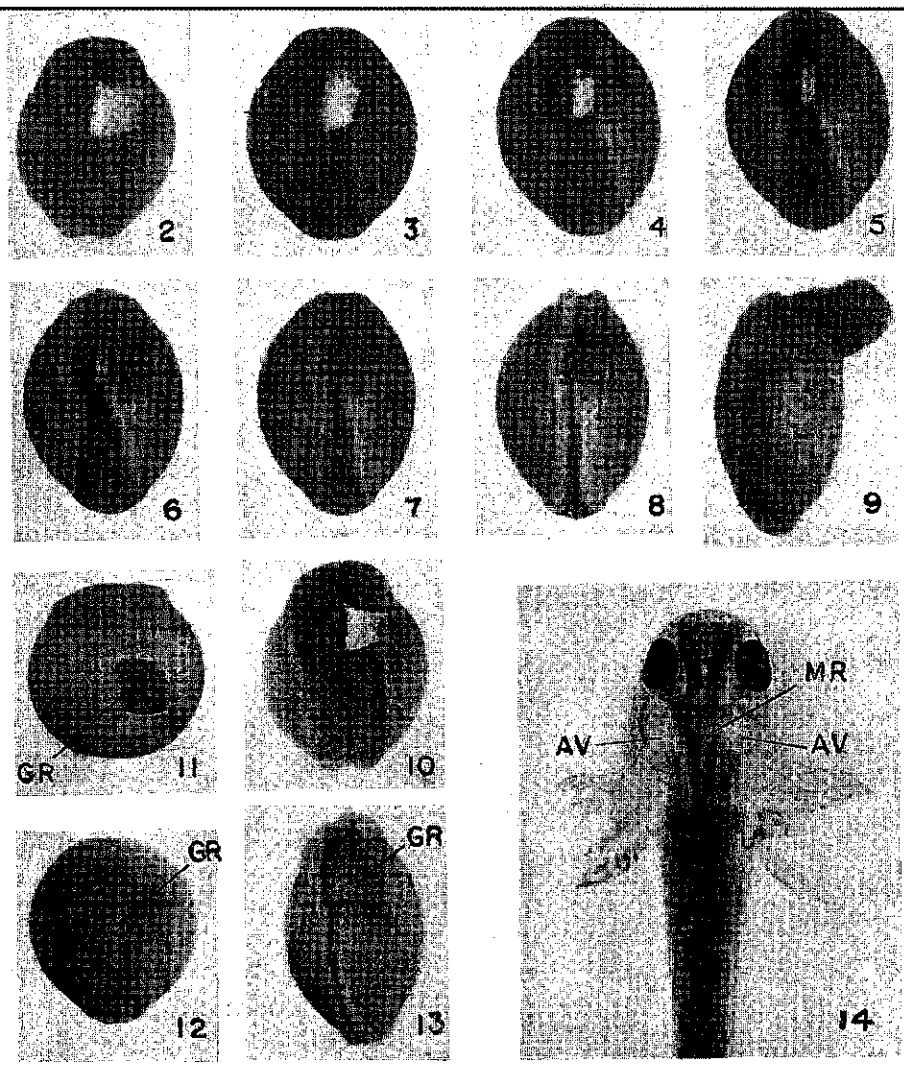

Figure 2. (2–9) Removal of right half of presumptive hindbrain with underlying mesoderm (Exp. RMP 119), wound left uncovered. (2) photograph taken 10 min after operation. (3) 30 min. (4) 50 min. (5) 1 hr, 10 min. (6) 1 hr, 30 min. (7) 2 hr, 50 min. (8) 5 hr, 40 min. (9) 48 hr, 25 min. (10) Removal of right half of presumptive hindbrain, mesoderm left intact (Exp. RMP 118), 35 min after operation; note difference in shape of wound from that in (3). (11–14) Removal of right half of presumptive hindbrain, mesoderm left intact, wound covered with ventral ectoderm, reciprocal operation. (11) Embryo from which the skin graft was taken, 1 hr and 50 min after operation (Exp. TrMP140). Dark patch (GR) is the grafted hindbrain and indicates the place of origin of the covering of the wound in (12). (12) Embryo from which half of hindbrain was excised, 1 hr and 50 min after operation (Exp. EMP 140); the graft extends laterally over part of the ear region. (13) Same embryo as in (12), 20 hr after operation; note the single neural fold and the spreading of the graft. (14) same individual (stage 46) with yolk completely resorbed; note completely restored hindbrain (MR) and ear vesicles (AV); specimen preserved and cleared. × 8.

thickened abnormal or a thinner layer of cells, the latter of which eventually develop normally).

- *Source of the original restitution materials* (logically, the material that makes up the defective hindbrain could come from the opposite normal side or from the damaged side. Observations are not conclusive by themselves).
  - ○ *Evidence from vitally stained material* (shows clearly that material comes from the opposite, stained, side).
  - ○ *Healing and reconstitution after bilateral extirpation of the hindbrain* (as the "opposite side" is also removed in this case, any restoration must come from the posterior or anterior edges. These cases yield no restitution of hindbrain).

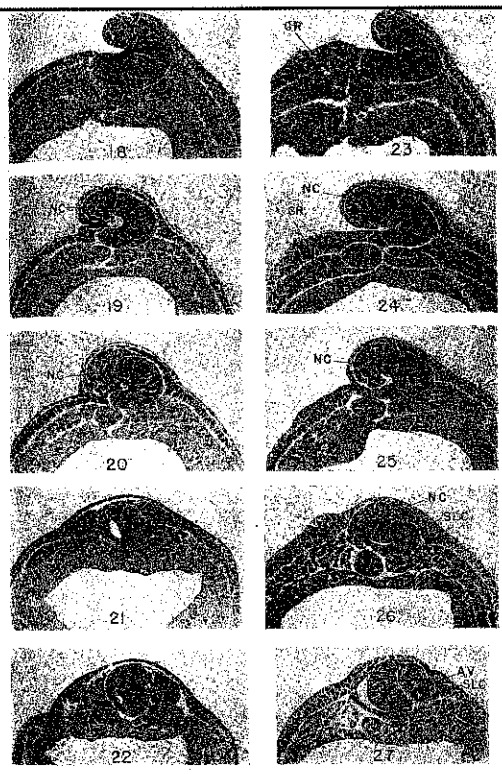

Figure 3. (18–20) Early effect of removal of half hindbrain; cross sections showing closure of single neural fold in cases in which the wound was left uncovered. (18) Embryo at stage 19, 12 hr after operation (Exp. RMP 30). (19) Embryo at stage 23, 24 1/2 hr after operation (Exp. RMP 29); the single neural fold touches the ectoderm covering the wound. (20) Same embryo section taken about 0.1 mm posterior to one shown in (19); here the ectoderm covering the neural fold has fused with the lateral ectoderm; neural crest begins to loosen up and migrate to each side. (21–22) Similar case (RMP 35) preserved 46 1/2 hr after operation (stage 28); neural tube completely closed. (21) Section through posterior part of ear region showing tube relatively thin walled on the defective side. (22) Same embryo, section through anterior part of ear region; fourth ventricle a narrow tube; mass of cells on roof; precursor of irregular or tubular type of healing. (23–27) Sections showing five early stages in healing and restitution of hindbrain in cases with wound covered by ventral ectoderm. (23) Embryo at stage 19, 14 3/4 hr after operation (EMP 57); neural tube wide open; graft (GR) much thicked. (24) Stage 21–24 1/2 hr after operation (EMP 52); opening into neural canal a narrow slit; neural crest (NC) begins to loosen. (25) Stage 23, 37 hr after operation (EMP 62); neural fold fused with ectodermal graft; neural crest migrating. (26) Stage 25, 37 hr, 40 min after operation (EMP 58); neural tube closed lying with its restored right wall ventral. (27) Stage 27, 60 hr after operation (EMP 63); neural tube closed and partially righted. For continuation see plate 3 (39–46). All figures are magnified 50 diameters.

- *Healing and reconstitution after unilateral excision of the whole cephalic portion of the neural plate* (Surprisingly, despite major damage, healing occurred as in the cases with lesser wounds. All parts of the brain and all sense organs develop, including the eye which had been highly debated, though the results are sometimes somewhat smaller or less symmetrical than in normal cases. Harrison clearly considered these results important, and developed complex modeling and detailed descriptions to show the results—described further below).
- *Resumé of evidence bearing on the origins of the original restitution material* (healing of a wound on one side results almost entirely from material shifting from the other side across the midline of the neural plate—contrary to previous conclusions by others.

- *Restoration of volume through hyperplasia after healing* (the amount of material involved varies, but hyperplasia takes place and can be measured and modeled, as seen in a series of graphs and tables. "The net result of these measurements is to show that, following excision of a lateral half of neural plate material, there is a restoration of the missing tissue to the extent that a quasisymmetrical structure is formed. This actually involves a large amount of hyperplasia, which affects mainly the defective side but also the intact side to the extent that the deficit brought about by wound healing and transference of material to the opposite end is made up" (Harrison, '47; p 61–62)).
- *Conclusion* (experimentally caused defects lead to healing in which "organisms make use of processes similar to those occurring in normal development as well as new ones where the normal do not suffice" (Harrison, '47; p 68). Neural plate material remains as a half tube, covered by a thin layer of neural crest folding over from one side only. In some but not all cases, the neural tube is able to close properly and develop normally, whereas other cases lead to a thick layer that does not allow proper closure. It seems to be mechanical accident that determines which will heal. There is also some evidence that damage stimulates cell division, "which after a time is most intense in the region of greatest defect, but which may affect other more distant regions where the defect is less, so that the end result is the restitution to approximately normal proportions" (Harrison, '47; p 71)).
- Here we might like Harrison to assign a cause or look for a mechanism to explain the phenomena, but he concludes that his observations "do not give any information as to what the nature of the stimulus to cell division may be." He concludes that the stimulus is not chemical as the tissues have not changed chemically, nor wound hormones or products from the damaged cells as they are sloughed off very quickly. Instead "It seems more likely that elastic forces are involved and that the abnormal stresses and strains brought about by defects in material may stimulate the remaining cells to more rapid cell division" (Harrison, '47; p 71). Harrison thereby

suggests that it is the three-dimensional arrangement of cells and tissues, and their relationships that are important.

- *Summary* (summarizes the experiments and results).

It is worth also noting the full range of tools that Harrison used to present his data and interpretations, and why he felt he needed such diverse approaches. Of course he showed the extirpation sites (his Fig. 1), results of excisions (his Fig. 2), and traditional cross-sections (his plates) to show both normal and experimental effects on neural tube and internal structure formation. In addition, he included graphs of data to show relationships between particular cuts and the resulting generated mass, numbers of cell divisions, and other details.

Beyond these more traditional modes of representation, Harrison also included a photograph of a wax model (Fig. 4—his Fig. 15). As Nick Hopwood (2002) has explained beautifully, wax models had become fairly common for medical education purposes by well before 1947, and Harrison had the advantage of a faithful assistant able to make such models. Sally Wilens made the models, apparently, and Harrison's illustrator Lisbeth Kraus made his drawings. Harrison explained that he had wax models made of three brains derived from experimental specimens. He photographed the whole specimens, took transverse cross-sections, then put the pieces back together again in a sense—creating a model to show the internal structure.

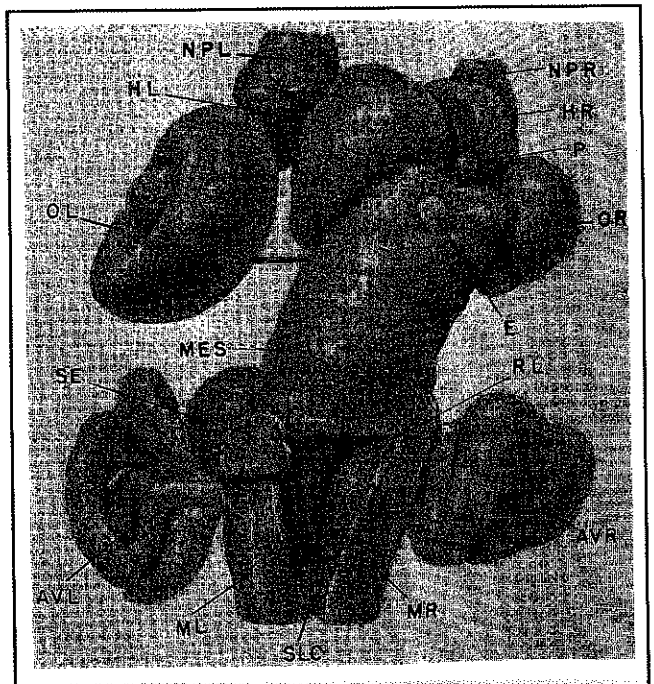

Figure 4. Wax reconstruction of brain and sense organs from a case in which the entire right half of the presumptive brain was removed (Exp. EMP 21); AVL, left normal ear; AVR, right defective (vesicular) ear; E epiphysis; HL, left normal hemisphere; HR, right regenerated hemisphere; MES, midbrain; ML, normal left half of myelencephalon; MR, regenerated right myelencephalon; NPL, left (normal) nasal pit; NPR, right nasal pit; OL, normal left eye with lens; OR, regenerated right eye; P, paraphysis; RL, recessus lateralis; SE, saccus endolymphaticus; SLC, sulcus longitudinalis centralis. × 50.

This was a lot of trouble. Why bother when this was not standard expected practice at the time? Apparently to show in 3-dimensional detail which organs responded in exactly what way to the experimentally induced neuroembryonic defects. He could show the exact amount and spatial orientation as the ear bent differently from normal, or that the eye was slightly out of alignment or the nasal pits slightly smaller but otherwise apparently normal, or that one bit of tube was a little smaller than the other. In wound repair, it matters a great deal whether what appears to be structurally normal really is. For understanding wound repair, it mattered a great deal to understand the relation of different parts to each other, and how a slight bend in one direction could affect the ability to regenerate the damaged part—or not. Harrison continually referred for comparison back to the normal series that he had established and that had been widely circulated to students and was published with his edited Silliman Lectures (Harrison, '69).

The experimental results appeared largely normal in structure and relations of the parts to each other. The details of the 3-dimensional structure made a difference in showing what was the same or potentially different from normal, given the constraints of his morphological experimental approach. His description and representations helped illuminate Harrison's understanding of the regenerative processes. Yet he concluded that "Further studies of the fiber tracts and nuclei should be undertaken with the use of specific neurological methods before sweeping conclusions are drawn with regard to the normality of finer structural details. It would indeed be remarkable if such a complex system should develop normally by this devious route" (Harrison, '47; p 72).

CONCLUSIONS

Similarly today: it would indeed be remarkable if the complex nervous system should develop normally by the "devious route" of stem cell regeneration and gene regulation. Possible, perhaps, but to what extent "normal"? That is the very important question. Harrison offered every bit of evidence he had at hand to show what he saw, what he knew, and how he knew it.

It is striking today that Harrison thought primarily in terms of structural and mechanical causes for this healing and system reconstitution. His results and discussions are all perfectly consistent with observations today, and they are highly suggestive about the possible underlying genetic and regulatory causes. The subtitle at the top of the pages following the first one is "Defect Regulation in Neural Plate," thereby emphasizing the regulatory aspects of regeneration or reconstitution, as Harrison also called it. Yet Harrison would himself never have imagined the impact of gene regulation on regeneration, because

throughout his career he did not see evidence about molecular or genetic effects at a level that could explain the phenomena at hand. For Harrison, genetics remained far too speculative and distant from the everyday needs of experimental embryology (Maienschein, '91a,b). So this is not a story about how Harrison knew it all a half century ago. It is instead a story about the underlying assumptions he made, and what cautionary notes follow as we think about assumptions today and how they constrain or inspire research.

Harrison's particular emphasis on experimental embryology, looking closely at morphological patterns and structural changes that he represented as empirically accurately as possible, was a standard of the time. By 1910, Harrison had become a leader in embryology, and by the close of his career with his final 1947 article he remained a towering figure. His particular work is therefore instructive as a type and as an example of a particular approach with particular assumptions.

Now, of course, regeneration researchers look more typically for up and downregulation, regulatory responses, knock-out, knock-down, RNAi, markers and indicators of signaling pathways, and other tools to help discover and interpret results of underlying genetic, molecular, and developmental causes of regenerative capacity. Such researchers may—but certainly do not always—also ask, as Harrison did, about what physical structures and mechanisms effect change under various different conditions. Or about the morphological relations among the resulting parts, or the 3-dimensional environment in which cells develop, for example, as stem cells move within the developing organism.

Stem cells may develop in the same "normal" way in a salamander or in artificial culture medium, as Harrison suggested in 1907. Wound repair may occur as cells move from their "normal" orientation to a wounded area to replace the missing cells and restore structure, as Harrison showed in 1947. Surely understanding the processes requires far more than the tools that Harrison had at hand, but reflecting on his approaches can help inform the choices and assumptions researchers adopt today in addressing the same fundamental driving questions about development.

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