Regeneration According to Spallanzani

Panagiotis A. Tsonis* and Timothy P. Fox

In this report, we elaborate on a letter that Spallanzani wrote to Bonnet reporting his findings on regeneration in worms, snails, tadpoles, and salamanders. The letter (original in French and translated in English; see Supplementary Material, which is available online) was written to discuss whether or not regeneration in these animals supports Bonnet’s theory on germs. The letter includes several drawings by Spallanzani, which were not published in the Prodromo, his book on Animal Reproduction. Spallanzani made important observations, which he described with considerable detail, but overall he was unable to confidently support Bonnet’s theory. This letter reflects the way of thinking in the 18th century that shaped the important scientific fields of regeneration and reproduction. Developmental Dynamics 238:2357–2363, 2009. © 2009 Wiley-Liss, Inc.

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INTRODUCTION

Rooted in Aristotelian philosophy, the belief that lower animals were generated spontaneously from decay prevailed until the 17th century when Redi in 1668 carried out well-controlled experiments that provided the first proof against it (Redi, 1668, 1671). A similar view, epigenesis, developed by Harvey, stated that new organisms appear from undifferentiated matter. These ideas were supported later in the 18th century by Needhan and Buffon who believed that organic molecules organized by internal molds were responsible for animal generation (Benson, 1991). The rival position to epigenesis, which prevailed at the end of the 17th century, was preformation. According to this, the organisms were preformed in the embryo. Preformation was also in favor when it came to religious beliefs because it fit the notion that all generations were established at the time of Creation. On the contrary, epigenesis allowed space for questioning the role of God. As expected when the first experiments in the 18th century revealed the regenerative power of animals, these two competing theories were called upon to explain this new property of animals.

The 18th century could very well be considered as the golden era in regeneration research. Many studies during the previous century had led the way to understand how reproduction occurs and many theories and thoughts were developed to explain how animals (and humans) reproduce (Cobb, 2006). Naturally, scientists experimented with regeneration in animals (which then was called actually reproduction). Pivotal discoveries by Réaumur on appendage regeneration in insects in 1712 (Wheeler, 1926), by Tremblay on Hydra regeneration in 1744 (Baker, 1952; Dawson, 1987), and by Bonnet on parthenogenesis in 1740 and on worm regeneration in 1744 (Savioz, 1948; Dinsmore, 1991) shocked the scientific world. Réaumur and Bonnet were preformationists and, in fact, Réaumur believed that germs were contained within parts responsible for regeneration. In his writings, Bonnet argued that Trembley’s experiments with Hydra and his with worms supported the pre-existence of germs and became a leading proponent of the preformation theory.

Among these intellectual giants, Lazzaro Spallanzani is also credited as being one of the pioneers in regeneration research. In 1768, Spallanzani published the Prodromo, his historical book on Animal Reproduction. At that time, the word “reproduction” was used to denote “regeneration” as well. In his book, Spallanzani described several types of regeneration with mention of regeneration of the frog tail and salamander limbs. The accounts were surprisingly quite short.

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with not much detail or any drawings that were common in publications of that time. However, in a long letter to Bonnet written on September 21, 1766, Spallanzani outlined in great detail regeneration in many organisms, including earthworm, snail, frog, and salamander (Biagi, 1958; Dinsmore, 1991). The letter, which reads as a research paper, is also decorated with many drawings by Spallanzani’s hand (original in French and translated in English can be found in Supplementary Material). One of the major reasons Spallanzani wrote this letter was to discuss Bonnet’s theory on reproduction and regeneration were mediated by germs that existed in the organism. Spallanzani initiated correspondence with Bonnet on July 18, 1765 by sending him copies of two of his publications. On August 24, 1765, Spallanzani wrote again to Bonnet critically commenting on Needham and Buffon’s ideas. Bonnet responded on September 14 and was quite happy that Spallanzani shared his views and that he was also working on earthworm regeneration. Bonnet in a sense invited Spallanzani to pursue the work on earthworm regeneration. An exchange of letters ensued in which Spallanzani outlined a series of experiments that were inspired by Bonnet’s influence. Bonnet mentioned that regeneration research should be able to resolve the germ concept, thus inviting Spallanzani to elaborate on this (Biagi, 1958; Dinsmore, 1991).

In his letter of September 21, Spallanzani casts doubts on the existence of germs for some types of regeneration and obviously he does this with great care not to offend Bonnet. Thus, this letter bears enormous significance about how scientists were thinking at that time and how they dealt with these obstacles imposed by their social/religious status as well as by the prevailing way of thinking of the time (for example, it questioned the Cartesian mechanistic view of animal biology or how the issue of soul fits in a regenerating animal). In addition, this letter also reflects the clarity of thought that Spallanzani possessed in order to explain his findings. We, therefore, strongly believe that the contents of this letter should be of great historical value to scholars in developmental biology and regeneration research. Here we will highlight his major experiments and points. Also, we have reproduced the drawings and we present them with added color as well. The color is in accordance with the description that Spallanzani provides in his letter. Figures 1–17 have been set in the order in which they appear in the letter, but have been grouped according to the species. We will end this review by reflecting on how these ideas shaped scientific thought in the 18th century and how they relate to our thoughts in the present day. Text in italics contains comments by us related to Spallanzani’s observations.

**Figs. 1–6.** Anatomy and regeneration in worms. **Fig. 1:** The gross artery (red) attached to the intestines. **Fig. 2:** The artery branches into five vessels (red) as it reaches the head. Spallanzani describes the vessels as small bags composed of one or many constrictions. **Fig. 3:** The vein that runs from head to tail is winding. It is intersected by another vein, which is straightened. **Fig. 4:** The “boat” worm, a fresh water worm, whose tail is on the surface of the water (light blue), while the head is hidden in the mud (dark blue). **Fig. 5:** Regeneration in fresh water worm. A cone is produced. The reddish color at the base of the cone is the anus. **Fig. 6:** At the posterior part of the anus, a red line appears in the same direction as the artery of the animal (o). Spallanzani describes that at this stage he could not see circulation in the red line but later blood flows into the artery.
REGENERATION IN EARTHWORMS AND FRESHWATER WORMS

After a short salutation and comments on how some scientists view Bonnet’s research, Spallanzani sets the stage by describing certain experiments he performed to learn about the anatomy and physiology of the earthworm. Spallanzani provides three drawings of the anatomy of arteries and veins (Figs. 1–3). He then proceeds by outlining six different experiments to study regeneration in earthworm. Drawings outlining these experiments and the results are shown in Supplementary Material.

**Experiment 1**

Transversal section of the earthworm into three parts in a way that the ovary, and the small bags (the means vessels; Fig. 2) remain in the anterior part, or in the part where the head is located. Results: After a few months, the posterior parts, or tails, and the middle parts all died, with the exception of 5 or 6 of them. The anterior part, which contained the ovary and the small bags, after 16 to 20 days started to generate a very small bud; this small bud stopped growing during winter, but continued its development during spring.

**Experiment 2**

Divide the worm transversally into 3 parts in a way that the ovary and the small bags belong to the middle part. Results: All anterior parts or the ends of the head died. The tails (posterior parts) also died with the exception of three of them. Many of the middle parts generated a head at their anterior extremity, and a tail at their posterior extremity.

Spallanzani attempts to provide some explanation of the few survivors that did not contain the reproductive system in experiments 1 and 2. Obviously, the pieces survived for several months and regeneration of the tail was more obvious.

**Experiment 3**

Remove from the worm the part of the head that remains above the ovary and the small bags. Results: All the ends of the head are dead but all the posterior parts containing the ovary and the small bags generated a head, which in the end was equal to the original one.

**Experiment 4**

Divide longitudinally the body into two parts from the head to two thirds of its length and then remove the posterior third (tail). Results: The two longitudinally divided parts and the tail part died.

**Experiment 5**

Divide the worm longitudinally into two parts from the tail to two thirds of its length and then remove the anterior part containing the head. Results: The two parts, which were separated, died, and the third part, which remained and contained the head, generated the tail (obviously the part contained part of or the whole reproductive system).

**Experiment 6**

Divide longitudinally the whole worm from the head to tail. Results: All the worms died.

Spallanzani then proceeds to discuss how earthworms grow. The question is: Is growth mediated by the addition of new rings or by the expansion of old ones? This question is related to preformation notions. In order to answer this question, Spallanzani decided to count rings in 11 worms that hatched from eggs and in 11 fully grown ones. He observed 84, 100, 96, 90, 102, 104, 88, 93, 96, 104, 90 in the former and 88, 94, 73, 101, 87, 83, 96, 80, 89, 95, 101 in the latter. Based on this, he concluded that growth is the result of expansion of old rings.

Spallanzani then turns to describe regeneration in another species, the fresh water worm. As for its appearance, he states that the part that contains the head is hidden in the mud, while the tail is pushed at the surface of the water forming a structure that resembles a groove or a boat (Fig. 4). He also describes the anatomy of the circulatory system for this worm. As with earthworms, Spallanzani experimented with regeneration. In one experiment, he cut 50 worms into two more or less equal parts. Almost all the anterior parts regenerated (obviously these parts contained the reproductive system) while most of the posterior parts (36/50) died. In the second experiment, he cut the worm into three parts in a way that the anterior part contained 3–6 rings and no reproductive system, and the middle part contained the reproductive system and the posterior part. At the end of the experiment, all the anterior parts were dead, the middle parts regenerated and many of the posterior parts were alive but without signs of regeneration. Spallanzani also mentions that at the level of the tail, the “boat” structure never failed to form, and he also concludes that these worms regenerate naturally because artificial regeneration (when he cut the worms) was identical with natural regeneration (when he found them in nature).

Having established that worms can regenerate, Spallanzani then attempts to explain the mechanism. Most of his observations are through the circulatory system, because this can be easily observed. First, he identifies a red dot by the edges of the anus. This shape of the anus stretches out. A cone starts to form, but the anus he claims is not at the tip of the cone but more at the rear of the cone (Fig. 5). At the posterior end of the animal (stump), a red line appears in the same direction as the artery of the animal (denoted as O artery in Fig. 6). In the beginning, the line does not show any circulation but later it does, and the blood flows into the O artery. Thus, the red line is the regenerative artery. The cone grows and the edges of the anus acquire more redness. As the cone grows, rings become obvious. Spallanzani also attempted repeated amputations, which were successful. He finally concludes that the shape (boat) is always formed and that the repeated amputations lead to shortening of the animal. Spallanzani does not elaborate here how germs could account for regeneration in earthworms, but he compares this later with head regeneration in snails. At the end of this section, Spallanzani develops some ideas about the respiratory system, but his observations are rather inconclusive. Despite the interesting way that earthworms regenerate and
the intriguing necessity of the reproductive system, which implies dependence on some specific factor, not much is known today at the molecular level. Nevertheless, after Spallanzani, many scientists experimented with earthworm regeneration. The necessity of certain segments, which include the reproductive system, has been verified (Morgan, 1901). In the earthworm, *Eisenia foetida*, which has 100 segments, amputation between segments 20–35 results in the failure of regeneration (Supp. Fig. 1). In this worm, regeneration is mediated by the formation of blastema, and ectoderm and endoderm maintain their identities. It also seems that annelids in general rely on dedifferentiation of older tissues, rather than pluripotent reserve cells (Goss, 1969). Nevertheless, studies on asexual reproduction in annelids have indicated the regeneration of primordial germ cells from parts of the body devoid of gonads, arising from piwi-expressing germile stem cells (Weisblat, 2006).

**REGENERATION IN GARDEN SNAILS (SLUGS)**

The next part of the letter deals with regeneration of head structures in snails. He first describes regeneration of the long horns (stalks), which "bear at their extremity a nice bud adorned by a blackhead."

Following partial sectioning of a horn, the rest of the horn gradually becomes rounded into a small bud, which grows and its color darkens. Finally, a dark spot appears at the tip of the bud. The bud grows, becomes longer, and after some time the mutilated horn acquires its normal length. Spallanzani notes that at the boundary between the old and the regenerated piece, there is a whitish color. When he cut both horns, both regenerated as well. Next, Spallanzani discusses the nature of the dark spots at the tip of the long horns. He disagrees with other authors that they are eyes. Rather, he believes that they are like antennas, which direct the snail during motion. Obviously, Spallanzani was wrong about this because it is now well known that the spots are eyes and their regeneration has been well documented (Eakin and Perlatte, 1973). But most amazingly, Spallanzani experimented with regeneration of the snail’s head! The severed pieces contained two lips, mouth, part of the esophagus, a tooth, muscles, and four horns (two long and two small ones). He observed regeneration in many of them (he used 200) after 26 days. He then attempted to compare these results with regeneration in earthworms. He attests that regeneration of the head from a sectioned posterior part of the earthworm is mediated by a cone, which is a whole organ, a miniature of a head waiting to fully develop. However, in the snail the head emerged in parts, which appeared one after another and reconstituted the head with time. Spallanzani describes one case where regeneration consisted of a bud made of two lips of the mouth and two small horns. The bud was "implanted" in the piece (the stump) (Fig. 7). The long horns were the next part that was regenerated. Spallanzani makes the case that not all parts are regenerating during the same time frame. He then proceeded to dissect the regenerated head and followed the direction of the four nerves that are inserted in the horns. He claims that he could not find where the old part united with the new one, and this was the case for the skin (outer surface of the horns) and esophagus. With these observations and the comparison he mentions with the earthworm, Spallanzani sets the stage for discussing Bonnet’s theory on germs.

Next, Spallanzani describes regeneration of the tail. He noticed that after amputation, the piece left behind retracts (Figs. 8 and 9). In the middle of the tissue (denoted as O in Fig. 9), there is formation of a “beak,” its color being white. That was in the center of the tail’s musculature and it continued to grow in all directions until it became equal to the removed part. The regenerated part maintained its white color and it was also recognized for having a non-stressed gross skin that is seen in the remainder of the
tail (maybe he is observing here a kind of wound epithelium, which has not become full-thickness skin). Even when the amputation was on an oblique surface, it was completed. Experiments with other snails, which he calls *Limaconi Ignudi*, were not as good in that they could regenerate the horns but not their heads.

Spallanzani then embarks on explaining regeneration based on Bonnet’s germ theory. As briefly mentioned above for earthworm and fresh water worms, Spallanzani is unable to explain it as “a simple extension”; therefore, he strongly believes that pre-existing germs must be involved. He based this on his observation that the cone at the site of amputation resembles the part that was removed. Even though Spallanzani tries to please Bonnet by supporting his theory, he cannot be quite sure that germs are responsible for the whole regeneration: “let me have a small doubt to your sound judgment.”

The doubt is cast from his observations that the gross artery, the two veins, intestines, and the musculature are so perfectly united in the reproduction (regenerated part), so that the parts are in continuity with the ones in the old animal. He discusses that he has difficulty seeing how germs can account for this. Likewise, he concludes the same for snail head regeneration because, as mentioned above, the parts regenerated separately and the regenerated nerves looked like extensions of the old ones. In order to gain more insight and information on this, Spallanzani turns next to his experiments on salamander and tadpole tail regeneration.

**TAIL REGENERATION IN SALAMANDERS**

First, Spallanzani describes tail regeneration in adult salamanders. After amputation, he observed a lot of blood flow and retraction of the skin at the edges of the stump. Then he observed that a kind of thin surface of reddish white color appear and the edges of the skin seem implanted on this surface. Spallanzani, in fact, describes here the formation of a wound epithelium (the thin surface). He then goes on to describe the beginning of a formation from the thin surface, which could be round or elliptic (what is known now as blastema). He observed that along the longer axis of the elliptic surface appears a thin crest of flesh, which is the beginning of the reproduction (regenerating tail). For anyone who has studied tail or limb regeneration, this is the early cone formation, which is most obvious at the center of the stump. Spallanzani in fact notices the different colors (a thin yellow line) between the old tail and the regenerating one (Fig. 10). He then describes how that central crest of flesh grows in all directions (clearly the formation of blastema) (Fig. 11). From the center of the regenerate, two small bands appear and they go up to the tip of the crest. These bands are brownish and the remainder of the regenerating tail is white (Fig. 12). In fact, Spallanzani notes that these two bands belong to two different planes in the regenerate. The bands that Spallanzani refers to are the regenerating spinal cord and notochord. After 3 months, the color has changed to become darker. Spallanzani
notes here that when he dissected the regenerating tail, it was very soft and he could not tell that the spine was made up of small bones. For him, this is an important observation because since he cannot see the small bones and the spine develops with time and is quite similar to the spine of the old tail, he concludes that simple extension cannot explain regeneration. Thus, this experiment would rather support development of germs. He then describes experiments where the whole tail was cut. He explains that for 26 days he saw no sign of regeneration. Eventually, the thin surface appeared. Then at each side of the spine two mounts of flesh appeared (ab and bc), which covered the spine (Fig. 13). There was some growth at the junction of these two mounts and not much more (Fig. 14).

Spallanzani correctly concluded that if too much of the tail is removed, regeneration is greatly retarded or inhibited. He then reports that in young salamanders (one month old), regeneration follows the same steps but is much faster. He also suggests that some bony small grains seen along the small band make the spine.

**REGENERATION OF TADPOLES’ TAIL**

Spallanzani begins by describing the gross macroscopic anatomy of the tadpole’s tail. He divides the tail in two parts, a major pointed one, which he calls “tongue,” and two lateral membranes (transparent tissue) (Fig. 15). In Figure 15, the tongue (light brown color) is demarcated by o, m, u, while the two membranes (yellowish-white color) are denoted by v, o, m and u, m, n. Inside the tongue is a kind of marrow (c, m; dark brown color) that runs across. This must be the cartilaginous spine because he even points out that it is not bony but is made up of a substance more consistent than the muscle. Because the tongue is still opaque, he cannot see circulation in it, but he can observe many small vessels running from the tongue to the lateral edges of the membranes and back. He then goes on to describe the circulatory organization in the tail and he points out that there are two main vessels. Here he disagrees with Leeuwenhoek who claimed to have discovered four vessels. After tail amputation, he describes two main events. One is the regeneration of the vessels and the other accumulation of “filaments” at the edges of the tongue. He points out that there are very remarkable differences between circulation in the natural part and the regenerated one (Fig. 16). In the former, the artery and vein make one vessel and run parallel to the length of the tail, but in the latter when the artery enters the regenerating part, it divides into many branches, which go on winding on each side toward the extremity of the tail, and then they turn upward, still being divided from each other. The division remains until they reach back at the amputation plane where they all unite into a vein. Older tadpoles also regenerated the tail but Spallanzani was unable to observe circulation. The same conclusions were made for the continuity between the old and new filaments. Also the skin that envelops the muscles of the regenerating tail was continuous with the old part, but had a different color (Fig. 17). He briefly mentions that regeneration of limbs in old tadpoles was never seen (obviously, he amputated them after a particular metamorphosis stage in which regeneration is not permitted).

The differences in regeneration (morphology) of the vessels allow Spallanzani to declare that tail regeneration in tadpoles originates from a lengthening (extension) of the old parts and not from development of germs. He also thinks that the way that the membranes regenerated gives more ground to his conclusion.

Spallanzani then tries very tactfully to elicit Bonnet’s opinion based on his results: “On which side is your inclination Dear Sir? In your very kind letter (the one Bonnet had sent to Spallanzani on August 8) you are nicely asking me if my observations confirm your principles. Now you can...
see with your own eyes and judge a thousand times better than I can.”

Spallanzani then states that Bonnet assigns two sources of repair. One that proceeds through the development of small filaments and explains regeneration of animal skin or plant peel and a second that involves the development of germs to explain regeneration of a whole organ or body parts. In a sense, Spallanzani attempts to tell Bonnet that germs cannot account for regeneration in snails and the tadpole’s tail. Spallanzani ends his letter confessing that to be certain he needs to do more experiments, especially to identify if he could observe the union between the germ (which he now calls an animal graft) and the stump. Spallanzani speculates that for circulation to be explained by germs (the regenerated part uniting with the old part, which results in common circulation), then a fossile of the graft should be found as in the case of the plant shunt. It is our impression that Spallanzani tries to leave room to support Bonnet’s theory.

Bonnet was a scientist who had learned not to succumb to dogmatism and thus he knew how to accept criticism. On January 17, 1771 he wrote to Spallanzani: “I have always sincerely sought the truth and I have warned a hundred times that I never flatter myself with the thought that I have always found it. So don’t spare me my errors, and criticize me whenever you judge it necessary. When nature pronounces against me you must not indulge in the language of friendship; and I will be the first to submit to its decisions.” (quoted in Savioz, 1948, and translated by Anderson, 1982).

THE PRODROMO

Two years later, in 1768, Spallanzani published the Prodromo, his book on Animal reproduction (Regeneration). Surprisingly, the book was quite short with not much detail or illustrations as he had suggested he would do. Nevertheless, it is considered to be a seminal publication for regeneration research. In the book, Spallanzani also describes regeneration of the limbs in salamanders. He noted that circulation in the regenerating limb is different than the original and that sometimes there would be cases with more or less bones. Even though he does not speculate, that observation also goes against germ theory.

CONCLUSIONS

It is obvious from studying this letter that Spallanzani based his conclusions on macroscopic observations made with different criteria. For example, several conclusions are based on the circulatory system, which could be hindered by the transparency of the tissue. To scientists in our time, it is easy to see where Spallanzani failed or succeeded in supporting Bonnet’s germ theory. Going against the preformation theory was a real moral problem for these scientists, because it had dominated their scientific beliefs and was rooted in their social status. The reader has only to consider that even today, when it comes to regenerative medicine and stem cells, opinions vary based on social status and religion. Notwithstanding this great problem, Spallanzani and the other regeneration pioneers made outstanding contributions to science and many of their observations were correct. Theories always are there to explain results. Even today, the mechanisms of regeneration are still debated. Is there true transdifferentiation or is regeneration mediated by undifferentiated stem cells? The newt has again been proven an indispensable tool. Studies conducted from the 19th century on have shown that dedifferentiation of somatic cells accounts for tissue repair during limb regeneration (Morgan, 1901). Thus, muscle, nervous tissue, and bone dedifferentiate to give rise to the regenerated muscle, nerves, and bone (Tsonis, 1996). During lens regeneration, the iris pigment epithelial cells from the dorsal iris form a continuous vesicle that then transdifferentiates to lens (Del Rio-Tsonis and Tsonis, 2003). If Spallanzani had seen histological preparations from these events, he would have concluded that regeneration is most likely explained through simple extension and he would have discounted germs. Likewise, if he knew all these marvelous examples of reserved stem cells or even the powerful potential of embryonic stem cells that can in principle build an organism, he might have written to Bonnet with joy that the germ theory is valid!

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