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Ernest Everett Just, Johannes Holtfreter, and the Origin of Certain Concepts in Embryo Morphogenesis

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SUMMARY

Ernest E. Just (1883–1941) is best known for his discovery of the “wave of negativity” that sweeps of the sea urchin egg during fertilization, and his elucidation of what are known as the fast and slow blocks to polyspermy. Just’s contemporary Johannes Holtfreter (1901–1992) is known for his pioneering work in amphibian morphogenesis, which helped to lay the foundation for modern vertebrate developmental biology. This paper, after briefly describing the life and scientific contributions of Just, argues that his work and ideas strongly influenced two of the concepts for which Holtfreter is best known: tissue affinity and autoneuralization (or autoinduction). Specifically, this paper argues that, first, Just’s experiments demonstrating developmental stage-specific changes in the adhesiveness of the blastomeres of cleavage embryos helped lay the foundation for Holtfreter’s concept of tissue affinity and, second, Just’s notion of the intrinsic irritability of the egg cell, which is evident in experimental parthenogenesis, strongly informed Holtfreter’s concept of the nonspecific induction of neural tissue formation in amphibian gastrula ectoderm explants, a phenomenon known as auto-induction. Acknowledgment of these contributions by Just in no way diminishes the importance of Holtfreter’s groundbreaking work. It does, however, extend the impact of Just’s work into the area of embryo morphogenesis. It connects Just to Holtfreter and positions his work as an antecedent to embryo research that continues to this day.

INTRODUCTION

For almost 40 years after the end of World War II, the work of E. E. Just, an African-American embryologist who is best known for his discovery of the “wave of negativity” that sweeps over the egg upon fertilization, lay buried in the scientific literature. Then, in 1983, on the one hundredth anniversary of Just’s birth, M.I.T. historian Kenneth Manning published a prize-winning biography (1983) that made accessible the life and work of Just, engendering a level of interest not evident before. Gould (1988) favorably reviewed Manning’s book, and soon afterward wrote a column in *Natural History* about Just’s scientific work (1985). Developmental biologist Scott Gilbert authored a book chapter (1988) comparing and contrasting Just and Richard Goldschmidt, and he cited Just’s discovery of the fast and slow blocks to polyspermy in his well-known textbook *Developmental Biology* (now in its eighth edition; Gilbert, 2006). Byrnes and Eckberg (2006) recently highlighted Just’s emphasis on the environmental contexts of development. *The Dictionary of Scientific Biography*, an important reference material for libraries, now has an entry on Just (Byrnes, 2007) whereas previously there was no entry. Finally, at least two universities have hosted symposia honoring Just and his work. The Medical University

of South Carolina has held a symposium in Just's honor each spring since 2001 and, in November 2008, a symposium honoring Just was held on the campus of Howard University (see www.eejsymposium.com). These activities indicate a renewed interest in Just and his work. Nonetheless, Just's contributions have not been fully appreciated. This paper, after briefly giving some biographical highlights and presenting some of Just's better-known experiments, will argue that his work and ideas strongly influenced the pioneering embryologist Johannes Holtfreter. Specifically, it will argue that (1) Just's experiments demonstrating developmental stage-specific changes in the adhesiveness of the blastomeres of cleavage embryos helped lay the foundation for Holtfreter's concept of tissue affinity in amphibian embryo morphogenesis, and (2) Just's notion of the intrinsic irritability of the egg cell, which is evident during experimental parthenogenesis, strongly informed Holtfreter's concept of the nonspecific induction of neuralization in amphibian gastrula ectoderm explants.

BIOGRAPHICAL HIGHLIGHTS

Born in 1883 in Charleston,¹ Ernest E. Just (see Fig. 1) spent his early years on James Island, a place rich in natural beauty located just off the South Carolina coast. At age 12 he enrolled at the Colored Normal Industrial Agricultural and Mechanics College at Orangeburg (now South Carolina State College), and at 15 he left the south entirely to attend Kimball Union Academy, a boarding school in Meriden, New Hampshire, from which he graduated in 1903. At 17, he enrolled at Dartmouth College in Hanover, New Hampshire, where he took up studies in biology, literature, history and the classics. Four years later, in 1907, Just graduated from Dartmouth *magna cum laude* (the highest honor bestowed that year) as an esteemed Rufus Choate scholar. He straightaway accepted a faculty position at Howard University, a predominantly African-American school in Washington, DC.

Just's first appointment at Howard was in the English Department but, because the university needed biology instructors, in 1910 he moved to the Department of Biology and Geology, where he taught zoology. He rose quickly through the academic ranks, becoming Associate Professor in 1911 and Full Professor of Physiology in the College of Medicine the next year. Later, with funding from a grant from the Rosenwald Foundation, Just established a Master's program in zoology at Howard and became Head of the first Department of Zoology. In 1915, he was chosen from among a group of distinguished nominees to receive the first National Association for the Advancement Colored People (NAACP) Spingarn medal for his excellence in research and his promotion of medical education. Just remained a faculty member at Howard until his death in 1941, despite many summer excursions to the Marine Biological Laboratory (MBL) at Woods Hole, Massachusetts, and, after 1929, research trips abroad to Italy, Germany, and France.

In 1909, through the recommendation of one of his college biology professors, Just contacted the renowned embryologist and director of the MBL at Woods Hole, Frank R. Lillie, who gave him a job as a summer research assistant. Thenceforth for 20 years, Just spent the better part of every summer to Woods Hole. Right from the beginning, his contributions were important ones. His first paper, in 1912, showed that the sperm entry point determines the first cleavage plane in the egg of the marine annelid *Nereis* (Just, 1912). This paper, Gould (1985) contends, "... already contain[ed] the basis for Just's later and explicit holism—his concern with properties of entire organisms (the egg's complete surface) and with interactions of organism and environment (the epigenetic character of development contrasted with the preformationist view that later development is already set

¹Unless otherwise indicated, the material for this biographical sketch was obtained from Kenneth Manning's excellent biography of Just, *Black Apollo of Science* (1983).

by the egg's structure)." With encouragement from Lillie, in 1911 Just enrolled in the PhD program at the University of Chicago, at first taking courses *in absentia* at Woods Hole and, later, in residence in Chicago. He received the degree 5 years afterward, in 1916. His dissertation work was concerned with the breeding habits of *Nereis limbata* and a related marine annelid, *Platynereis megalops*, as well as the fertilization reaction of the sand dollar *Echinarachnius parma*. His subsequent work at Woods Hole focused mainly on the fertilization reaction and the structural changes that occur on or just below the egg cell surface during the fertilization process (see Scientific Contributions Section).

While at Woods Hole, Just rose from student apprentice to respected scientist. In 1929, however, he made his first foray abroad to do research. It was to the Stazione Zoologica in Naples, Italy, for 6 months. While there, he studied the fertilization reaction in several European sea urchins, and showed that the Mediterranean annelid *Nereis dumerilii* was not the same as the closely related species at Woods Hole, *P. megalops*, as others had proposed. Just's second trip came soon after his first. In early 1930, he received an invitation from the famous biologist Max Hartmann to visit the Kaiser-Wilhelm-Institut für Biologie in Berlin, of which Hartmann was the director, for 6 months. The Germans had been following Just's work closely, and they were interested in seeing if his ideas about the importance of the cell cortex could be extended to unicellular protists such as *Amoeba proteus*, which is easy to study because of its large cell size, and other *Protozoa*.

Altogether, from his first trip in 1929 until his last in 1938, Just made nine trips abroad to pursue his research interests. Several were to Berlin, but a number were to Naples and to Paris, where he spent time at the Sorbonne completing his second book, *The Biology of the Cell Surface*, in 1938. Just published several papers from his 1929 studies of the marine animals in Naples (Just, 1929a,b), and after the 1930 trip to Berlin, he published several papers in German scientific journals, including one in *Naturwissenschaften* correlating changes in cell adhesiveness with developmental stages during early cleavage in starfish embryos (Just, 1931). Two papers that sought to present a cytoplasm-centered alternative to the existing gene-centered account of mutation and the origin of evolutionary change followed soon thereafter (Just, 1932, 1933). After 1936, Just's papers became much more philosophical as he tried to apply his ideas on the importance of the cell surface to biology more broadly (Just, 1936, 1940). His book *The Biology of the Cell Surface* (1939b) brought the complete body of his scientific work as well his more general ideas together as one synthetic whole.

Penniless; bereft of funding for his research; and rejected by American scientists for his strong opposition to their reductionist views of biology, his public snubbing of his colleagues at Woods Hole (see below), and his love of European science and women (he had two affairs with German women, eventually marrying the second, Hedwig Schnetzler, after divorcing his wife and leaving his family), in 1938 Just initiated a self-imposed exile in Europe. At first he and Hedwig lived in Paris, but later they moved to the Station Biologique at Roscoff, a small fishing village on the English Channel. However, in 1940, the Nazis began their siege of Paris and the surrounding countryside, including Roscoff. Just was briefly interned in a camp for prisoners, but was released with the help of Hedwig's father. He returned to Washington and Howard University, where he resumed his faculty position and his research activities. (His new wife Hedwig settled nearby in New Jersey.) In 1941, Just became gravely ill with pancreatic cancer. Shortly after the beginning of the fall semester, he died. He was only 58 years old.

JUST'S PHILOSOPHICAL INCLINATIONS—ORGANICISM

Just's writings reveal that he embraced the philosophy of organicism, or materialistic holism, which posits that cells and organisms are complex wholes that are "inherently greater than the sum of their parts" (Gilbert and Sarkar, 2000). The properties of any level of organization (molecule, cell, tissue, organism) depend on the properties of the parts of the level below, as well as the properties of the whole into which they are integrated. Properties emerge out of the organizational complexity of the system. Stephen Jay Gould describes this philosophy as the "middle position," lying as it does between vitalism on the one hand and mechanism on the other: "The middle position holds that life, as a result of its structural and functional complexity, cannot be taken apart into its chemical constituents and explained in its entirety by physical and chemical laws working at the molecular level. ...Life must be studied at its own level; its complexity must be tackled directly, not dissolved into components—for the interaction of these components is primary and irreducible" (Gould, 1985). Gould concluded that Just was an organicist, and this conclusion is borne out in Just's writings, all of which show that Just believed that life arises out of the complexity and structural integrity of living systems. In *The Biology of the Cell Surface* he wrote "[L]ife is the harmonious communion of events, the resultant of the communion of structures and reactions" (Just, 1939b). Moreover, "[we scientists] overlook the organo-dynamics of protoplasm—its power to organize itself. Living substance is such because it possesses this organization—something more than the sum of its minutest parts" (Just, 1933). This philosophical view places Just squarely in the company of other classical embryologists of his time. However, unlike others, he was willing to publicly take on such giants as Thomas Hunt Morgan, the Nobel laureate who proposed that genes on chromosomes are the units of inheritance, in his defense of the cell's holistic integrity. At the 1935 American Society of Zoologists meeting in Princeton, New Jersey, for example, Just challenged Morgan, and presented his own (clever but ultimately incorrect) cytoplasm-centered theory of how differentiation occurs during development. His "theory of genetic restriction" stated that "...all the differences, i.e., differentiation, that appear during development, rest upon cytoplasmic reactions. These are made possible through removal of obstacles by nuclei, hence, by chromosomes and genes. The nuclei by removal of substances release the activity of the cytoplasm in one direction" (Just, 1939b). Unfortunately, if Morgan was too nucleocentric, Just was even more dramatically off the mark. Nonetheless, Just's explanation, though incorrect, reveals a pronounced egalitarian and de-centralized view of the cell. This view certainly is becoming more common today with the rise of epigenetics (Van de Vijver et al., 2002).

SCIENTIFIC CONTRIBUTIONS

Altogether, Just published more than seventy scientific articles over a 30-year period, from 1911 to 1941. The journals in which he published included *Science*, *Naturwissenschaften*, *Protoplasma*, the *American Journal of Physiology*, *Biological Bulletin* and *American Naturalist*. He wrote two books, *Basic Methods for Experiments on Eggs of Marine Animals* and *The Biology of the Cell Surface*, both published in 1939. His scientific contributions, discussed below, lay in several areas: the breeding habits of marine invertebrates; the fertilization reaction and the "fertilizin" theory of fertilization; the fast and slow blocks to polyspermy; and experimental parthenogenesis.

- *Breeding Habits of Marine Invertebrates*: Some of Just's earliest work involved investigating the breeding habits of the marine invertebrates *Nereis limbata* (clam worm) and *Platynereis megalops* (Nereid worm) that lived off the sea coast at Woods Hole. The two papers he published on this work, one which described the mating behavior of *N. limbata* (Lillie and Just, 1913) and the other which described the egg-laying rituals of *P. megalops* (Just, 1914), contributed to his PhD

dissertation. Fascinating to read, these papers showcase Just's powerful writing ability. They also highlight his strong naturalist tendencies, and his growing awareness of the importance of environmental factors in development. They reveal Just's staunch belief that the experimental embryologist should become intimately familiar with the processes of fertilization and development as they occur in nature. Later, in his methods book describing the proper handling of marine animal eggs and embryos, *Basic Methods for Experiments on Eggs of Marine Animals* (Just, 1939a), Just emphasizes this point. "[T]he experimental embryologist should as far as possible know his animal personally and directly through work in the field, never resting content to become what Kropotkin in another sense denominated a 'desk biologist'." By the late 1920s, Just's knowledge of the natural history of the marine animals at Woods Hole had become legendary. Many scientists at Woods Hole and beyond, both junior and senior, sought his advice. Whereas others often failed, Just invariably succeeded in coaxing marine invertebrate embryos to develop normally. He compiled a set of "indices of normal development," based mainly on when and under what conditions fertilization membrane separation occurred. These allowed him to predict with precision whether or not development would be normal for a given egg.

Just believed that eggs should never be treated as raw material. "The cell is never a tool," he wrote. It is a living system and not a machine that can be used to "prove a theory" (Just, 1939b). It is this view of biology that led Just to clash with prominent reductionists such as Jacques Loeb (see below) and T. H. Morgan (above). But this view, which is holistic, integrated and systems-oriented, also resonates with increasing numbers of biologists today (see Newman, 2003; Byrnes and Eckberg, 2006). Moreover, three characteristics—Just's deep knowledge of the natural settings in which fertilization and development occur, his insistence that laboratory conditions closely match those in nature, and his conception of the organism as an integrated system with properties that emerge out of its complex organization—reveal a close affinity between Just's work and an emerging field of biology known as ecological developmental biology (Byrnes and Eckberg, 2006). See Gilbert and Epel (2009) for an up-to-date and thorough treatment of this field.

- *The Fertilizin Theory of Fertilization*: Frank R. Lillie, Just's mentor at Woods Hole, had proposed that the mature egg of a female member of the species *Nereis* or *Arbacia* gives off a diffusible substance called "fertilizin" that attracts sperm and causes them to adhere to the egg cell surface. The fertilizin molecule was proposed to have two ends, one that binds to a receptor on a spermatozoon, and the other that binds to a receptor on the egg. Unfortunately, Lillie's model shared much in common with Paul Ehrlich's side-chain theory of immune action, which had fallen into disfavor at the time. As a result, it did not fare as well as Jacques Loeb's rival theory of fertilization, which proposed that fertilization was mediated by changes in ion concentration in the egg initiated by the introduction of a lytic "sperm factor" into the egg. Just nevertheless was able to show that Lillie's fertilizin theory held true not only for *Nereis* and *Arbacia* (Just, 1919a) but for other marine animals as well (*Platynereis*, *Echinarachnius* and others). In 1930, he wrote a strong defense of the theory (Just, 1930). He may have been motivated by a sense of loyalty to his mentor, but also, as Gilbert (1988) points out, by the fact that the proposed site of production of fertilizin was the cell cortex (ectoplasm) and Just was very interested in highlighting anything that gave the ectoplasm a larger role in fertilization. We now know that both Lillie (and Just) and Loeb were partly correct. Indeed, as Parrington et al. (2007) point out, our understanding of the process of egg activation today is still couched in terms of the two alternative models of Lillie (and Just) and Loeb.

- *Fast and Slow Blocks to Polyspermy:* Using a light microscope, Just was able to observe in detail the structural changes that occur at the egg cell surface and in the layer below it, the cortex or ectoplasm, during fertilization. As early as 1919, he observed that “[b]efore the actual elevation of the [fertilization] membrane, some cortical change beginning at the point of sperm entry sweeps over the egg, immunizing it to other sperm ...” (Just, 1919b). He emphasized that it was this “wave of negativity” sweeping over the egg at the onset of membrane separation, not membrane separation itself, that was responsible for the block to polyspermy. Thus, Just is credited with being the first to infer the existence of what is now known as the fast block to polyspermy, a phenomenon that subsequently has been shown to be caused by a shift in egg cell membrane potential (see Gilbert, 2006). Although he did not directly observe the fast block—he could not have done so because it is a nearly instantaneous electrochemical event—he did recognize that a phenomenon by which additional sperm are blocked from entry into the egg (a fast block) does exist. He also observed the slow block, a mechanical one, which occurs as a result of formation of the fertilization membrane (or envelope). He wrote: “As the membrane lifts off, it carries away any supernumerary sperm whose activity is in contrast to immobilized sperm previously engulfed by the egg.” Moreover, he noted that membrane elevation at any given point prevents sperm entry at not only that point, but at every other point on the egg surface (Just, 1919b). It is his inference and his detailed documentation of the fast and slow blocks to polyspermy for which Just is best known (Gilbert, 2006). However, as this paper emphasizes, he made important contributions in other areas of embryology as well.
- *Experimental Parthenogenesis:* While at Woods Hole, Just investigated the effect of a number of variables (hypo- and hypertonic sea water, UV irradiation, temperature) on the artificial activation of eggs in the absence of sperm, a phenomenon known as experimental parthenogenesis. The animals whose eggs he studied included species of *Echinarachnius*, *Nereis*, *Platynereis*, *Arbacia*, and *Chaetopterus*. His work here led to the longstanding scientific disagreement he had with Jacques Loeb, whom Stephen Jay Gould labeled “the great reductionist of American embryology and physiology” (Gould, 1988). Prior to their disagreement, Loeb had recommended Just for the prestigious NAACP Spingarn medal (mentioned above) but afterwards he described Just as being “limited in intelligence, ignorant, incompetent, and conceited” (Manning, 1983). Part of Just’s disagreement with Loeb was based on the fact that Loeb was extremely reductionistic; Loeb believed that, by tapping into the power of parthenogenesis, humans could gain control over nature and engineer her to their benefit (Pauly, 1987). But another part of the disagreement was because Loeb sometimes was not careful in his experimental technique. As a result, Just believed that Loeb had reached conclusions that were not valid. In contrast, Just was extremely scrupulous in his own work—a perfectionist, really—carefully controlling for variables such as temperature and evaporation. Indeed, for Just, if the eggs used in an experiment were not 100% normal, then all results obtained with them were suspect. Under such compromised conditions, the results obtained could be due to the “poor physiological condition of the eggs” and not the variables being tested (Just, 1939b).

As early as 1900, Loeb had shown that he could induce parthenogenesis in eggs by exposing them to hypertonic sea water. However, irregularities arose in the embryos that subsequently developed, so that only a small fraction developed to the larval stage. Loeb further discovered that by treating the eggs with a solution of butyric acid followed by hypertonic sea water, a much higher percentage of parthenogenetic embryos developed normally to the larval stage. This became known as Loeb’s double treatment method or, as Just called it, his “superficial

cytolysis-corrective factor” method of parthenogenesis. Loeb’s method was very popular, but Just succeeded in systematically tearing it apart. First, he showed that only one part of the two part treatment—either butyric acid or hypertonic sea water—was needed. Second, he showed that the cytolytic effect of the butyric acid was merely a function of the egg’s having been overexposed to the acid. Third, he proved that the order of treatment was inconsequential. He wrote: “Thus, the sequence in the treatment so strongly demanded by the ... theory not only is not supported by fact but is contradicted by it” (Just, 1939b). What Just viewed as the important feature of experimental parthenogenesis was very different from what Loeb viewed as important. For Loeb, the action of the nonspecific agents (ions and acid) upon the egg, causing it to begin to develop, was the critical feature. In contrast, Just’s focus was not on the agents (sperm, ions, acid) that triggered development. Rather, his focus was on the egg itself. For him, what was all-important was the egg cell’s responsiveness to its environment. He called this property of the egg its *independent irritability*, which he believed extended to all living cells and tissues. This intrinsic responsiveness was mediated through the cell surface. As will be argued below, Just’s notion of the intrinsic responsiveness of the egg cell to outside agents strongly influenced Johannes Holtfreter, a pioneering embryologist known in part for his discovery of the process of autoinduction, in which nonspecific agents can artificially induce explants of amphibian gastrula ectoderm to form neural tissue.

JOHANNES HOLTFRETER

Johannes Holtfreter (1901–1992; see Fig. 2) was an icon in the history of research on embryo morphogenesis. Between 1929 and 1955, he virtually single-handedly elucidated the role of what is known as Spemann’s “organizer” in the inductive patterning of the amphibian embryo, work that lay the foundation for much of today’s vertebrate developmental biology (Gerhart, 1996). The organizer had been discovered by Hans Spemann and Hilde Mangold in 1924. In their now-famous experiment, when they grafted the blastopore lip of the early amphibian gastrula into the ventral marginal zone (presumptive belly skin region) of another gastrula of the same age, it initiated gastrulation and embryogenesis in the surrounding tissue, causing the formation of a two conjoined twin embryos (Spemann and Mangold, 1924). Holtfreter, a member of Spemann’s laboratory at the time, also began working on the organizer. In 1931, he invented a special “Holtfreter’s medium,” a balanced salt solution, and introduced sterile techniques. These improvements dramatically opened up new avenues for experimentation on embryos and embryo fragments. Through work over many years, Holtfreter was led to conclude that the organizer operates “by evoking or actually derepressing developmental possibilities latent in the competent surrounding tissues, which then self-organize details of their later development without the organizer’s further intervention” (Gerhart, 1996). He also developed maps of differentiation capacity for the salamander early gastrula, or as he called them “competence maps,” by systematically transplanting small pieces of tissue from one part of the gastrula to another. From the early 1930s on, Holtfreter and many others embarked on an arduous search for the “inducer,” the agent given off by the organizer that induces surrounding tissue to differentiate. This effort, which lasted for three decades, eventually “petered out and ended, in the 1960s, on a note of despair and resignation” (Holtfreter, 1991). Only recently has work on the organizer been revived (see De Robertis, 2006).

The search for the inducing agent led to the confusing discovery that “a great diversity of foreign tissues, chemical substances, and environmental conditions ... can perfectly imitate the action of what we may refer to as the ‘genuine’ inducing agent” (Holtfreter, 1991). Even “chemicals off the shelf” were found to evoke neural development by the salamander

gastrula ectoderm (Gerhart, 1996). The effort to find the mysterious endogenous inducer was dealt a final blow when Barth (1941) showed that even low pH can trigger neural induction. Holtfreter confirmed and extended Barth's experiments. He proposed that artificial inducing agents worked by causing a "sublethal cytolysis" of the cells, and that this near-death experience is what led to the subsequent differentiation of aggregates of the cells upon removal of the inducing agent (Holtfreter, 1947). Holtfreter argued that the brief exposure to the injurious environmental conditions "caused a liberation or activation of a cell-intrinsic physiological process that steered the cells, after their recovery from shock, into neural differentiation" (Holtfreter, 1991). Thus, Holtfreter's emphasis was on the inherent responsiveness of the ectoderm cells. As will be argued below, Holtfreter's concept bears great similarity to a concept proposed by E. E. Just—that of the inherent responsiveness, the "independent irritability," of the sea urchin egg to nonspecific, artificial agents during experimental parthenogenesis.

In another set of groundbreaking experiments, Holtfreter (1939) discovered that when separate tissue segments derived from different germ layers of an amphibian embryo are recombined in vitro, they form a composite mass made up of distinct cell groups in which differentiation can take place. Apparently, the cells sort themselves out, reorganizing according to not only the topography they would have had in situ, but also the timing of morphogenetic structural changes (Grunwald, 1991). Thus, Holtfreter was observing developmental stage-specific changes in cell adhesion. He proposed that these changes in cellular adhesion play a critical role in not only embryo morphogenesis, but also in the overall evolution of multicellular organisms (Grunwald, 1991). As we will see shortly, E. E. Just earlier had correlated changes in the adhesiveness of the blastomeres of cleavage-stage starfish embryos with cell surface structural changes (Just, 1931). Below, it will be argued that this earlier work by Just (and others) helped prepare the groundwork for Holtfreter's later discovery of tissue affinity.

THE JUST–HOLTFRETER CONNECTION

Scott Gilbert writes that although Just's work showed an important role for the cell surface in development, it was "met with polite neglect. Although his 1931 paper in *Naturwissenschaften* (Just, 1931) had provided the first evidence for functional changes in the cell surface during development, it was all but ignored, and even when cited (as in [L. V.] Heilbrunn's books), it was not discussed. When research on the cell surface began again after World War II, Just's work was quickly forgotten" (Gilbert, 1988). Echoing Gilbert and emphasizing Just's contributions in the area of cell adhesion research, Gerald Grunwald, in his historical account of embryonic cell adhesion research, makes a similar point. He writes that the aforementioned work by Just, which was concerned with the cleavage reaction of the *Asterias* (starfish) egg, "can be cited as the first experiment demonstrating developmental changes in the adhesiveness of cells" (Grunwald, 1991). In *The Biology of the Cell Surface* (1939b), Just describes this elegant experiment involving the four blastomeres of the two-stage cleavage egg of *Asterias*:

Normally during early cleavage the blastomeres of this egg lie within the vitelline membrane apart from each other; later, regaining contact they develop into one embryo. When after the second cleavage the four blastomeres lie apart, they may with care by puncture of the vitelline membrane be removed as four independent cells. If brought together again and kept in close contact ... they unite and develop into a single embryo. Also, two blastomeres from one egg when brought together with two from another in some cases united and developed into one embryo; often however union failed to take place. I found that this failure resulted whenever the transferred blastomeres were not in exactly the same moment of development. ... Observation revealed that the surface-changes in the transposed blastomeres were

different. ... The failure of blastomeres from two eggs of the same fertilized lot to unite therefore is to be attributed to changes in ectoplasmic [cell surface] behavior resulting from a difference in rate of development which is seconds only.

Here, Just has discovered that the ability of the cells to come together, that is, their adhesiveness toward each other, is exquisitely developmental stage-dependent. To what extent might the work of Johannes Holtfreter's on tissue affinity in the amphibian embryo (Holtfreter, 1939) have been influenced by this discovery of Just's?

In 1939, the year his paper was published, Holtfreter was in the throes of escaping Nazi Germany. He found refuge in England, and then was shipped to Canada, where he was held in an internment camp for 2 years. Released in 1942, he made his way to McGill University and, in 1946, took a faculty position at the University of Rochester, where he stayed until his retirement in 1968 (Gerhart, 1998). Earlier, in 1935, Holtfreter had spent a year in the United States, including some months at the MBL at Woods Hole, where Just earlier had worked under Lillie's mentorship. It had been 5 years, in June, 1930, since Just had been at Woods Hole for the last time, never to return again despite the fact that he had spent summers there for almost 20 years. The occasion of that last visit was Frank Lillie's 60th birthday. Manning (1983) poignantly describes the scenario. After giving a short talk describing his recent experiments, Just stepped off the podium and said: "I have received more in the way of fraternity and assistance in my 1 year at the Kaiser-Wilhelm-Institut than in all my other years at Woods Hole put together." Manning (1983) writes that Just "left Woods Hole without saying goodbye to anyone, not even Lillie. He never returned." One can only speculate about how this incident may have impacted Just's relationships with his colleagues at Woods Hole. Moreover, what might Holtfreter have been told about Just during Holtfreter's visit five years later? What effect might this sad and unfortunate incident, as well as Just's increasing outspokenness about his own ideas about biology, have had on others, especially Holtfreter?

As documented in Manning's book (1983), the 1930s was the period when Just began to take more frequent and longer trips to Europe. His ideas about the important role of the cell surface (the ectoplasm) were well received in Germany, and he began to feel more comfortable around European scientists. He began to take a more philosophical view as he strove to make a mark on biology more generally. He became more confident and forceful. Arguing in favor of the holistic, cytoplasm-centered view of the classical embryologists, he took on giants in American biology, including Jacques Loeb (who died in 1924) and Thomas Hunt Morgan. As Gilbert (1988) has written, Just became an "outsider" to American science. His view on the importance of the cell periphery rather than the nucleus contrasted sharply with the emerging nucleocentric views of the American geneticists. Gilbert describes Just's view of the cell (and the ideal society) as egalitarian as opposed to authoritarian. "The nucleus did not give any orders to the cytoplasm. Rather, the order was coming from the entire embryo. ... The cell was not ruled by the nucleus; for that matter, it was not ruled by the cytoplasm either. The cytoplasm was far more important than the nucleus to be sure, as it contained the developmental potentials and reacted with other cells and with the external environment; but the total community of embryonic cells is what determined the fate of a particular cell" (Gilbert, 1988). What is interesting in this regard is that, although Hamburger (1996) has characterized Holtfreter as reductionistic—an understandable characterization given Holtfreter's propensity for taking embryos apart and studying their pieces in vitro—John Gerhart has this to say:

Although [a] cell-centered view is now taken for granted in cell and developmental biology, it was a rare and penetrating view in Holtfreter's time. From our vantage point of fifty years, it seems as if Holtfreter brought to light an individualistic and anti-authoritarian view of the embryo in which competent responsive cells interact

in a self-organizing community, in place of conceptions of the embryo as a collection of naïve passive members dependent for their future on detailed directions from a central organizer (Gerhart, 1998).

If we substitute “nucleus” for “organizer” or vice-versa, these statements about these two scientists’ views sound very much alike. For both, the embryo is a self-organizing community of responsive cells. Thus, we see that Just and Holtfreter may have shared deeply similar ideas about the nature of cells and embryos and their innate responsiveness to environmental factors.

What is ironic is that it may have been the warm reception Just received during his 6-month stay at the Kaiser-Wilhelm-Institut für Biologie in Berlin in the first half of 1930 that contributed to his bitter assessment of Woods Hole in June of that year. Just had been invited to Berlin by Max Hartmann, the prominent German biologist. While there, he developed collegial relationships with not only Hartmann, but also Richard Goldschmidt and Otto Mangold. According to Manning (1983), Just was especially close to Holtfreter, who was Mangold’s assistant at the time: “Life at the Institut was informal, comfortable, homey. On the way to his own table, located in Hartmann’s *Abteilung* on the third floor, Just would stop for coffee and a chat with scientists in the *Abteilungen* run by Mangold and Goldschmidt. ... Just especially liked talking with ... Holtfreter, who had fascinating ideas about painting and sculpture as well as fertilization and development.” Just and Holtfreter corresponded regularly after Just’s return to the United States. How might the collegial relationship between Holtfreter and Just have changed during the 9 years between Just’s visit to Berlin in 1930 and the 1939 publication of Holtfreter’s paper on tissue affinity in embryo morphogenesis? One can only guess, but Holtfreter’s lack of attribution to Just is noticeable. It is interesting that many years later in an interview with Aaron Moscona, Holtfreter made this comment. “The idea of tissue affinities grew out of some earlier work that I had done with amphibian embryos, *but it was not entirely original*. In 1939, I wrote a short paper on this and I have been surprised by the attention it started to receive” (Moscona, 1986, cited in Grunwald, 1991; emphasis added). Might this have been an attempt by Holtfreter to finally acknowledge (if obliquely) the contributions of others, including Just?

In the above discussion, one can see a connection between Just’s experiments showing developmental stage-dependent changes in the adhesive properties of cleavage embryo blastomeres and those of Holtfreter on cell adhesion in the amphibian embryo. But there is another area in which the connection between Just’s work and Holtfreter’s is even stronger. In 1948, Holtfreter presented a model for the phenomenon of autoneuralization (autoinduction), in which explants of the amphibian gastrula ectoderm develop into neural tissue when subjected to a variety of nonspecific agents such as calcium-free salt solution or distilled water (see also earlier discussion; Holtfreter, 1948). Holtfreter drew a parallel between the phenomenon of autoinduction and that of experimental parthenogenesis, in which an egg cell is nonspecifically activated by an agent (hypo- or hypertonic sea water, butyric acid) other than a spermatozoon (see above). Years later, in an essay reflecting on his life’s work, Holtfreter wrote:

The above results of mine [i.e., nonspecific induction] indicated that the treatments merely operated like an unspecific trigger, setting in motion a preexisting, pent-up mechanism which, through unknown chains of events, led to neural differentiation. I therefore referred to this phenomenon as “autoinduction.” However, how could it be explained that such a diversity of environmental interferences elicited the same end-result—neural differentiation? ... Perhaps, so I thought (Holtfreter, 1948), the situation is analogous to the one in experimental parthenogenesis of the sea urchin egg, where a variety of exogenous chemical and physical stimuli can imitate the action of the penetrating spermatozoon, namely, to activate egg development. What

these activating agencies seem to have in common is that they change the structure and permeability of the plasma membrane. This, in turn, would entail the same kinds of physiological and morphogenetic chain reactions that occur in the normally fertilized egg (Holtfreter, 1991).

By way of comparison, here is what Just wrote in *The Biology of the Cell Surface* about his work on experimental parthenogenesis:

As we have already seen, the nature of the experimental means is not specific. Most probably, the nature of the reaction between experimental means and egg differs also between spermatozoon and egg, since there is evidence to indicate that fertilization is a chemical union of an egg-substance with the spermatozoon, whereas we can assume that the initial action of the experimental means is physical. But the end-result, however reached, is the same. The conclusion is therefore this: the egg-cell like many another living cell—nerve or muscle, for example—possesses independent irritability. It has full capacity for development. Neither spermatozoa nor experimental means furnish the egg with one or more substances without which the initiation of development would be impossible (Just, 1939b).

Just firmly identified the egg surface (cortex) as the epicenter of the cell's intrinsic responsiveness, and noted that debasement of the cortex can lead to nonspecific effects. "This independent irritability of the egg in my experience at least ... is largely due to its ectoplasm or cortex. Specificity in fertilization depends upon the integrity of the cortex ... It would seem logical to assume, therefore, that the egg cortex plays the leading role in fertilization which is the peculiar manifestation by the egg of its irritability" (Just, 1930). As mentioned above, the key to autoinduction (and induction) in amphibian gastrulae is the intrinsic responsiveness of the tissue that receives the inducing signal, its competence to respond. Likewise, the key to the activation of the egg during parthenogenesis (or fertilization) is the responsiveness of the egg to activating agents, its independent irritability. For Just, independent irritability was an inherent property of all living cells, tissues and organisms. He related it directly to the "organizator" theory: "Strictly orthodox morphologists have likewise often presented theories of life-processes on the basis of demonstrations that only emphasize anew the capacity of the living thing though debased to respond according to its specific and intrinsic irritability. The now perfect collapse of the organizer theory is a case in point: all that remains of it is a name for the well-known power of protoplasm to respond in the same characteristic, structural and physiological manner to diverse stimuli" (Just, 1939b). Interestingly, despite the clear parallels between Holtfreter's work and Just's, Holtfreter cited Just in only one place in the 1948 paper. That citation was to experiments showing the inhibitory effect of "coelomic fluid" on the fertilizability of *Arbacia* eggs! Given the strong parallels between Holtfreter's concept of autoinduction and Just's concept of intrinsic (or independent) irritability, the attribution given seems grossly misplaced and inadequate.

The reasons for Holtfreter's lack of acknowledgement of Just's contribution to his theory of autoinduction may never be known. They are likely complex, and rooted in science, culture, and personal history. And, in all fairness, it appears that Holtfreter was in general not always forthcoming in his recognition of others' work. For instance, Grunwald (1991) notes (in the context of the aforementioned tissue affinity work) that "[t]he initial analysis of vertebrate embryonic cell adhesion in its modern form has been traditionally attributed to Johannes Holtfreter, beginning with his 1939 paper [on tissue affinity] ... However, although certainly aware of the aforementioned studies by H. V. Wilson, Lillie, and Just, Holtfreter makes no reference to these studies in the introduction to his work." It should also be added that there is no evidence that Holtfreter harbored any kind of prejudice against Just because of his

race; on the contrary, the evidence is that Holtfreter was respectful of different cultures and ethnic groups as a result of his having traveled broadly in Southeast Asia and elsewhere around the world (Holtfreter, 1991).

Whatever the reasons for Holtfreter's lack of citation of Just, the fact that Just did contribute to Holtfreter's concepts of tissue affinity and autoinduction highlights the current relevance of Just's scientific legacy. It places Just's contributions where they belong: within the distinguished tradition of research on embryo development. Just's contributions are thus a direct historical antecedent to embryo research being done today. Tokindo Okada, in his account of his and Masatoshi Takeichi's discovery of the calcium-dependent cell adhesion molecules known as cadherins, describes his excitement upon reading (in 1949 after some delay in its availability) Holtfreter's (1939) paper on tissue affinity: "The paper was a real revelation to me. It proposed great perspectives for explaining the entire complex process of embryonic morphogenesis in a unified fashion by introducing the term *Affinität* (affinity)" (Okada, 1996). By contributing to Holtfreter's groundbreaking work on tissue affinity, Just's experiments on the differential adhesiveness of cleavage-stage blastomeres indirectly informed the experiments that led up to the discovery of cadherins, as well as the work that has been done since that time. Likewise, by informing Holtfreter's concept of autoinduction, Just's notion of the intrinsic responsiveness of cells may have contributed to the eventual realization that it is the *competence* of tissues to respond to an inducer of development that is critical.

As an aside, moving ahead from the late 1940s when Holtfreter first formulated these ideas to the present time, it is interesting to note that the molecular basis of the phenomenon of autoinduction has been uncovered only recently. Just 2 years ago, Hurtado and De Robertis (2007) showed that neural induction in the salamander *Ambystoma maculatum* (the same species Holtfreter studied) by agents as nonspecific as sand particles in the absence of organizer is mediated by the Ras/MAPK signaling pathway. These results contrast with earlier ones (Wilson and Hemmati-Brivanlou, 1995) suggesting that the differentiation of ectoderm to neural tissue that occurs during autoinduction may be the result of dilution of endogenously produced inhibitory bone morphogenetic proteins (BMPs) when the cells become dissociated as part of the procedure. According to this explanation, which is based on what is known as the "default model" of neural induction (Hemmati-Brivanlou and Melton, 1997), it is the removal of inhibition by BMP that allows the cells to differentiate. However, Kuroda et al. (2005) recently showed, in experiments using *Xenopus* animal cap cells, that BMPs continue to signal even after dissociation of the cells. Thus, neural plate development is a much more complex process than was originally envisioned (Stern, 2006).

CONCLUSION

Ernest E. Just, an early 20th-century African American biologist of international standing who is best known for his elucidation of the fast and slow blocks to polyspermy, made important contributions to a number of areas of biology: the breeding behavior of marine invertebrates, the fertilization reaction in sea urchin and other marine animals, and experimental parthenogenesis. But the impact of his work extends also into the area of embryo morphogenesis. Specifically, his work appears to have influenced the work of the pioneering embryologist Johannes Holtfreter. First of all, Just's experiments demonstrating developmental stage-specific changes in the adhesiveness of the blastomeres of cleavage embryos helped lay the foundation for Holtfreter's concept of tissue affinity in amphibian embryo morphogenesis. Secondly, Just's notion of the intrinsic irritability of the egg cell, which is revealed during experimental parthenogenesis, strongly informed Holtfreter's conception of autoinduction. The acknowledgement of Just's contributions to these concepts of tissue affinity and autoinduction is important, for it connects Just with Holtfreter and

places Just's work in a direct line with all that came afterward. Giving due credit to Just is important also because the same oppressive forces that stymied his achievements also militated against his receiving proper recognition. His fellow American scientists used his insights but they often did not cite his work. They may have felt justified in doing this because Just was not well liked in America; they may have felt they could get away with it because Just was black. He was not a member of the scientific establishment; he had no recourse to fight back. Such historical examples of the unfair treatment of one scientist by others certainly are not unique to Just's situation. But it is still important to set the record straight.

Frank Lillie, Just's friend and mentor at Woods Hole who stood by him until the end, wrote a moving obituary of Just in *Science* the year after he died. In it, he said: "... That a man of his ability, scientific devotion, and of such strong personal loyalties as he gave and received, should have been warped in the land of his birth must remain a matter for regret" (Lillie, 1942). James F. Crow, in a charming and accessible essay on the life and work of Just, writes: "How I wish Ernest Just had been born a century later" (Crow, 2008). Indeed, how much more might Just have accomplished had he been born later, when segregation and racist attitudes had diminished? Yet his achievements stand on their own merit. The fact is that he *did* make important contributions despite the oppressive social and cultural milieu that existed. Moreover, one could argue that the insights he had about the workings of nature were deeply informed by his unique life experiences. Remove the experiences and the insights vanish. Ultimately, science is a human and a social endeavor. Great discoveries and insights in science are often informed by experiences and images from the scientist's life. Gilbert (1988) has suggested that Just's strong gravitation toward holism, his emphasis on the importance of the cell surface as opposed to the nucleus, was in large part a manifestation of his being African-American in the early twentieth century. If Just had not experienced discrimination and rejection in the United States, he would not have spent so much time in Europe, and he would not have embraced the more holistic, integrated view of European biologists. In the end, what is singularly impressive is that, in the midst of crushing inequalities and, ironically, also *because* of them, Just made unique and significant contributions to biology that continue to resonate today. These lasting contributions are a testament to the brilliance and the indomitable intellectual ferocity of Just.

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Figure 1.
E. E. Just, circa 1920. Alfred Huettner, photographer. Courtesy of the Marine Biological Laboratory Archives.

Figure 2.
Johannes Holtfreter, circa early 1950s. Courtesy of University of Rochester Library,
Department of Rare Books and Special Collections.