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REVIEW

Ray Owen and the history of naturally acquired chimerism

Aryn Martin

Department of Sociology, York University, Toronto, Canada

ABSTRACT
This article interweaves a history of Ray Owen's early work with a broader account of the conceptual landscape of immunology in the mid 1950's. In particular, Owen's openness to the very possibility of chimeric phenomena is recognized.

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In 1945, a short article in *Science* simultaneously laid the conceptual foundations of acquired immunological tolerance and launched Ray Owen's research career.[1] In the article, Owen reported that fraternal twin cows often have 2 distinct blood groups: their own and that of their twin. Several decades earlier, F. R. Lillie had shown that dizygotic twin cows often share blood circulation in utero.[2] It was well known, on farms at least, that the female twin of a mixed-sex pair was often sterile (a "freemartin"). Lillie showed that their prenatal blood supplies are connected by anastomoses, and that subsequent exposure to masculinizing hormones caused the sterility. The crux of Owen's interpretation, meticulously demonstrated in more than 80 pairs of bovine twins, was that more than hormones circulate between twin cows. Blood and haematopoietic cells must do so also, allowing mixed bloods to persist into adulthood. In Owen's words, "the critical interchange is of embryonal cells ancestral to the erythrocytes of the adult animal. These cells are apparently capable of becoming established in the haematopoietic tissues of their co-twin hosts and continuing to provide a source of blood cells distinct from those of the host, presumably throughout his life."[1]

Louis Pasteur famously said "in the field of observation chance only favors the prepared mind." While serendipity will factor in this story (as in most biological surprises) Owen's background and outlook made him uniquely qualified to make the contribution that he did. In addition to the first finding of spontaneous chimerism in animals, Owen foreshadowed the field of maternal-fetal microchimerism in a 1954 publication,[3] planting a seed that would have a long germination. As readers of this journal know, natural chimerism and its role in health was a field that would not flourish for almost 50 y. Owen's 1945 publication on cattle twins was an important piece of the puzzle of immunological tolerance, which would shortly thereafter be articulated by Burnet and Fenner.[4] It is an irony that these same luminaries proposed the theory of self/nonself distinction: this theory would, in the latter half of the twentieth century, become a field-governing dogma[5,6] that made researchers *less prepared* to observe and appreciate chimeric phenomena. Philosopher Alfred Tauber argues that "the historical development of the discipline [immunology] reflects a deep-seated conceptual orientation to an individual-based biology at the expense of a more comprehensive interactive ecology."[5] In recent decades, incontrovertible evidence of natural and ubiquitous chimerism has contributed to a dawning appreciation of immune system complexity, revealing the limitations of a model committed to genetically homogeneous selfhood. Once considered dangerous to the health and survival of organisms, "others" in the form of genetically nonself cells (along with commensal bacteria[7] and other symbionts[8] too) are increasingly understood as important components of the biological self, whatever that may be. One suspects that this would be no surprise to Ray Owen.

CONTACT Aryn Martin aryn@yorku.ca 2150 Vari Hall, 2700 Keele St., Toronto Ontario M3J 1P3, Canada.

Before his widely-known work on cattle twins, Owen first published as an undergraduate at Carroll College in Wausheka Wisconsin. His article[9] gives us a window into this young man's character and sensibilities on the cusp of graduate school and hints at why he was poised for a finding that would become a touchstone not just in the history of acquired immunological tolerance but in the unfolding of the story of natural chimerism as well. Owen won a national essay competition with his entry "The place of the study of biology in the educational program of the United States." His own assessment of this piece was characteristically modest: "It was the work of a callow young undergraduate in a little, relaxed college. And it was on a pretty dull subject."[10] By his own admission, though, "the article attracted a little attention" and helped propel him toward graduate school at the University of Wisconsin in Madison. Owen's undergraduate essay makes a sustained case for the importance of biological education, an enterprise to which he subsequently devoted many decades of his life. Owen's reasons for the personal rewards of biological knowledge are particularly eloquent and prescient:

> Biology both arouses and satisfies the element of wonder in our natures. It presents life as a whole, a great general plan of which individual living things, plants and animals, are simply modifications. It places a supreme value upon life, painting all life as a great net in which the life of each individual is a delicate strand. It cautions its students that the destruction of any of these precious strands will disturb the whole net.

Given the ecological challenges we face 70 y later, one wishes that Owen's sensibility of the delicate interconnectedness of all life was more widespread. In this passage and elsewhere in the essay, Owen depreciates self-interest and underscores the interconnectedness of all living beings. While other concrete intellectual and practical preparations made him uniquely poised to appreciate the significance of his future observations, this sensibility of life's delicate entanglement was also essential. Like all subsequent researchers of natural chimerism, Ray Owen was open to the possibility of cells living outside their genetic organism, an orientation requiring that one must not see the individual as a closed and autonomous system.

The major contours of Ray Owen's life and work have been featured in recent obituaries and reminiscences[11,12] as well as in histories of acquired immunological tolerance.[13,14] In order to provide a somewhat different angle I will focus here on some lesser-known details and anecdotes, many of which were told in Owen's own words to Rachel Prud'homme, an interviewer for the Caltech Oral History Project. Prud'homme spent many hours over 5 d in 1983 drawing out recollections and impressions from the newly emeritas researcher, devoted teacher and veteran administrator. The transcript[10] reveals an eloquent, witty, humble and generous man, and paints a picture of his readiness for the part he would play.

A young life on the farm prepared Owen for his later work with cattle and all manner of experimental animals. Several early teachers recognized his promise as a scholar, while others assumed he was destined to adopt the more likely vocation of a farmer. He recalls one high school teacher urging him to take a foreign language to prepare for college while another said "What the hell do you expect him to do, swear at the cows in French?"[10] Owen enrolled in Carroll College, a small liberal arts school that enabled him to commute from the farm. In his senior year, while president of the Pioneer Club, he moved to Pioneer House on campus. He paid for it by selling Fuller Brushes in his "little old Model T-Ford," meeting all sorts of folks in Depression-era midwestern America. His good fortune at Carroll College included the aforementioned essay prize, an awakened passion for biology and genetics and, most formatively, meeting his life-long partner June.

After college, the couple moved to Madison where Owen began graduate work in L.J. Cole's lab. He describes Cole as "very permissive about what you might choose to do and how you chose to do it."[10] This style—where students were not tethered to a specific advisor's projects, or to any particular species and materials—would become a hallmark of Owen's later style of supervision when he ran a lab of his own. Openness to ideas, species, materials, techniques and hunches seems to have paid off in Owen's intellectual development and outputs, and he sought to cultivate this freedom in his students. In the interview with Prud'homme, he reflects on this style:

> [I]n my lab, I never adopted the principle that grad students should work on a problem for which I was getting research grant support and be components in some kind of machine, all of them working on the same highly focused area. I don't mean to imply that the way I structured things is better than other ways. But it does have some advantages, because each graduate student had his

> or her own material and problem and area, often things that they had dreamed up for themselves, and we talked over and tried very diverse kinds of things.

During his own days as a graduate student, Owen was involved in diverse collaborative and independent projects ranging from the biochemistry of pigeon irises to chromosomal translocations in rats.

Owen's thesis work on the hybrid offspring of crosses between guinea fowl and domestic fowl sought to explain why these progeny were often sterile.[16] While an investigation into sterile hybrid animals seems an obvious precursor to his later work with freemartins, the connection that Owen himself makes is telling in a less obvious—though probably more important—way for a history of microchimerism. He explains that germ cells in birds originate outside the embryo and migrate from the circulation to the genital ridges, and then become the primordial germ cells of the gonad. "And because the problem dealt with these migrating cells, when I later encountered the phenomenon of erythrocyte chimerism in bovine twins it was natural for me to think about how that could have happened, because I was already familiar with cell migration in embryonic development."[10] Chance favors a prepared mind indeed.

Following his PhD completion in 1941, Owen joined the immunology and genetics laboratory of M. R. Irwin, where the primary work was with dairy cattle. The commercial context for Owen's later discovery is an interesting element in the story. Irwin, along with a veterinarian collaborator Lloyd Ferguson, had connections with a number of American purebred cattle associations for whom they performed pay-by-the-test lab work. In essence, they were doing paternity testing using blood group characteristics to ensure valued lines of inheritance. "It made our research possible, because we got blood samples from all over the world—they'd bleed whole herds of cattle, and cow and bull families, and send the samples to us for our tests and studies. And they funded our work."[10] It was in this connection that Owen and his collaborator Clyde Stormont had noticed "something funny" about twin calves. When twin brother and sister—obviously fraternal because they were unlike in sex—were tested for blood groups, they appeared identical. Like most biological surprises, the case that made Owen's career came about through some serendipity. A farmer in Maryland wrote to Owen to say that he had some cattle twins that Owen might be interested in studying. In cows, fraternal twins are sometimes the product of insemination by 2 different bulls. In this case, the first was a planned dalliance with a purebred bull, a Guernsey like the mother. Later the same day a fence-crasher, this time a Hereford bull, bred the cow again. The offspring, a brother and sister, were clearly only half-siblings: one was a purebred Guernsey and the other a Hereford-Guernsey hybrid with the dominant white-face marking of the Hereford breed. In Owen's blood workup the twins tested as though they were identical, and both cows contained blood groups from both fathers.

Owen had the skill and ingenuity to develop a technique—later known as differential hemolysis—that enabled separation of the 2 red-cell populations in each twin. He found that the twins had an intermixture of blood, and deduced that it was the result of the shared circulation already described by Lillie. Because the life-span of bovine red cells is about 120 d and these twins were a year old, Owen explained, "what I was seeing was more than just a mixture of red cells, a transfusion. Essentially a transplantation had occurred between these twins, so that blood-forming cells deriving from each twin had settled out in the hematopoietic tissues of the other."[10] Owen followed up this case by testing in "some hundreds" of other fraternal twin combinations (including same-sex pairs and products of 2 rather than 3 parents) and found that the phenomenon was the rule rather than the exception; it occurred in about 90 percent of cases. Interestingly, the proportion of bloods in both cows was always around the same. In other words, they weren't mirror images, as one might expect if the exchange was reciprocal, but each cow had the same degree of mixture as the other cow.

Owen sent a long paper to *Science* describing the finding, and tantalizingly "foreseeing the possibility of what was later to be called immune tolerance."[10] The journal said the article was too long, and a much shorter version appeared as the now-classic paper "Immunogenetic Consequences of Vascular Anastomoses between Bovine Twins." The paper caused little stir at the time, but was picked up and cited in 1949 by Australians MacFarlane Burnet and Frank Fenner as evidence of a "unique natural example" of the phenomenon they named "tolerance." This phenomenon they placed in the context of their "self-marker" hypothesis,[4,14] which would become ascendant in biomedicine as the "self/non-self" theory whereby an organism recognizes self and actively defends against pathogens and tissues that are not self.

Meanwhile in Britain, biologist Peter Medawar and his young associate Rupert Billingham had a cattle mystery of their own. Initially unaware of Owen's work, they undertook what they thought would be a simple technique by which you could differentiate identical from fraternal twin cows.[14,16] Their prediction, from like studies in humans and in other species, was that identical twins would readily accept skin grafts from each other while fraternal twins would reject them, just like any other sibling pair. To their surprise, all cow twins incorporated the foreign tissue without immune rejection.[17] It was only when Medawar became aware of Owen's work, while reviewing Burnet and Fenner's book in 1950, that he could explain the confounding observations. Further experiments ensued whereby Medawar, Billingham and Leslie Brent worked out the details of acquired immunological tolerance.[18] In 1960, Burnet and Medawar shared the Nobel Prize for Physiology or Medicine. Incidentally, Medawar wrote to Owen to say that he believed it was very wrong that Owen was not included in the Prize. Owen repeatedly demurred, expressing embarrassment whenever the conversation arose.

Cows weren't involved in every surprise in the history of chimerism. In 1953, British physician Ivor Dunsford found, during routine testing, that a healthy blood donor named Mrs. McK had 2 separate blood types, O and A. Dunsford enlisted blood grouping expert Robert Race of the MRC Blood Group Unit in London to make sense of the mystery. In a letter to Medawar, requesting some advice on the unusual case, Race wrote: "We thought of the cattle story and suggested that they ask Mrs. McK if she were a twin, and to everyone's surprise she said she was."[27] The woman's twin brother had died as a young child and about half of her blood sample was composed of cells derived, from some sort of *in utero* exchange, from her brother's genetic lineage. Given the frequent sterility of female twins in a mixed-sex pair of cattle, the researchers involved were quick to query Mrs. McK's fertility. In his first letter to Dunsford, Race added in postscript: "I suppose Mrs. McK is not obviously a freemartin—has she been pregnant I wonder."[28] To this, Dunsford replied: "I am told by Dr. Bowley that Mrs. McK is a 'femine female' [sic] with a sufficient quota of curves and bumps to attract and wed a spouse and bear him one child."[29] The story of Mrs. McK appeared in the *British Medical Journal* titled "A human blood group chimera."[20,21]

It is of historical significance to note that Owen did not originally call the phenomenon "chimerism," though he readily adopted the moniker once it had become established in the UK. His term for the situation in fraternal twin cows was "erythrocyte mosaicism." The evocative term chimaera had first garnered a biological meaning (rather than mythical or literary) when German botanist Hans Winkler used it to describe his inter-species plant amalgam of tomato and nightshade tissues in 1907.[22] Medawar and his colleagues, in their 1951 article about the cattle twin skin transplants, tweaked its meaning to refer also to same-species combinations found in nature. Their definition of chimaera, "an organism whose cells derive from 2 or more distinct zygote lineages," has informed the present meaning of the term.[18]

It did not sit well with Owen's Wisconsin colleague Clyde Stormont that Race adopted Medawar's term instead of Owen's. The Wisconsin lab must have received a pre-publication draft of the article. Stormont wrote to Race in defense of *erythrocyte mosaicism* on the grounds that Owen had gotten there first. In the letter, Stormont gripes about the "inevitable problem which often seems to follow when English researchers write on subjects developed first by American investigators."[30] In a response that exudes Owen's graciousness and good humor, he quickly deflated the issue, copying the letter to Race:

> Fact is, Clyde, though I deeply appreciate your willingness to take up the cudgel for EM, I don't feel that the nomenclatorial issue here is a profound one; I regard it as something of a chimaera (var. of chimera, a monster vomiting flames; a horrible illusion; a vain or visionary conception). If you are right in accusing our English colleagues of toying with our terminology, I suspect that part of the explanation might lie in their generally more sensitive discrimination in the use of English; for myself, I have long envied the writing of Englishmen. I rather wish I'd thought of chimera first myself; it is a somewhat more specific term than mosaic and, as far as I can see, almost entirely appropriate to the situation … I have an impression of incongruous juxtapositions when I see the term chimera … quite different from the neatly matched elements of a mosaic.[31]

Race replied lightheartedly: "I am sorry [Stormont] is getting a bit excited about the word chimera (as the BMJ insists on spelling it). My only thought in using it was to get a good title for the paper."[32]

Owen's deference in this matter is in keeping with his amiable commitment to collegiality in science. Granted, he may have been leery of participating in an acrimonious dispute about nomenclature. Indeed, a "prolonged and often bitter" controversy had been going on between UK scientists Robert Race and R. A. Fisher on one hand and, on the other side of the Atlantic, American Al Weiner.[22] They disagreed about the proper understanding of Rhesus blood groups in humans, and thus used different symbols to represent the groups. In his interview with Prud'homme, Owen referred to this dispute, explaining "only now …. do we find that there was really truth on both sides—as is true in most bitter polemics, I think. The situation didn't justify the heat that incomplete knowledge invested in it."

Owen's diplomacy paid off in lifelong good relations with his British colleagues. It is an intriguing thought experiment, though, to imagine the fate of chimerism or microchimerism had they been named something else. As is well known, the Chimaera is a mythical monster whose main attribute is, in Owen's words, incongruous juxtaposition of multiple species (a lion, goat and serpent to be precise). Ostensibly this is an apt name for genetically distinct cells in a single organism which—according to the tenets of self/nonself immunology—are indeed incongruous, uncanny and (nearly) fantastical. Only since the 1990s when Thomas Starzl et al.,[23] William Burlingham[24] and others entertained the possibility of long-term chimerism in transplant recipients, and Diana Bianchi,[25] Lee Nelson[26] and others demonstrated the phenomenon in women and their children, have we come to see that from the body's point of view, the juxtaposition of genetically disparate cells might not be so incongruous after all. In the 1950s chimeric phenomena seemed exceptional and rare. Contemporary evidence suggests that they are ubiquitous.

This brings me back to Ray Owen's part in maternal-fetal microchimerism. In 1954, Owen and colleagues published a study with evidence that Rh negative daughters of Rh positive mothers develop a degree of tolerance to Rh antigens because of *in utero* exposure (see Kinder et al. this issue). Owen et al. wrote: "In multiple births of cattle an apparently similar tolerance is associated with the establishment of intact cells, interchanged between embryos. If the present hypothesis stands, it would be of considerable interest to determine whether the maternal Rh antigen or *intact maternal cells* mediate a similar acquired tolerance."[3] What explains the decades-long gap between this invitation and fulsome appreciation of maternal-fetal cell exchange? Technical capacity such as PCR for one thing. It is also plausible that the paradigmatic status of self/nonself boundaries eclipsed attention and research into microchimerism. Part of having a prepared mind, then, is being able to conceive of something inexplicable and apparently contrary to the laws of nature. This was Ray Owen's gift.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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