Perspectives

Anecdotal, Historical And Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

A Golden Anniversary: Cattle Twins and Immune Tolerance

James F. Crow

Genetics Department, University of Wisconsin, Madison, Wisconsin 53706

OCCASIONALLY a chance observation jump-starts a whole field of science. The discovery of immune tolerance and the recognition of self and nonself is such an event. It started with a 1944 letter from a cattle breeder in Maryland to the University of Wisconsin immunogenetics laboratory, reporting a curious pair of twin calves, unusual in having different fathers. RAY OWEN, a postdoctoral fellow in the laboratory and already interested in blood groups of cattle twins, thought they would provide an interesting opportunity for blood group analysis, so blood samples were sent to him.

In the thirties and forties, the genetics of blood cell antigens was an active field for investigation. It was then a popular view among geneticists that antigens, because of their simple inheritance, might be immediate gene products. For this reason, they might provide an insight into the nature of that maddeningly elusive entity, the gene. In pursuit of this possibility, new blood types were actively sought in various species, including *Homo sapiens* and *Bos taurus*. By the early 1940s, 40 different antigenic specificities had been identified in cattle.

The world leader in cattle blood groups was the immunogenetics laboratory at the University of Wisconsin, founded by L. J. COLE and M. R. IRWIN (OWEN 1989). There was sometimes uncertainty about paternity in cattle, and when valuable animals were involved, that could be an important economic issue. Breeders and breed associations welcomed blood groups as a foolproof way of identifying sires. The immunogenetics laboratory provided valuable information and the breed associations provided financial support, vitally important in those pre-NIH/NSF days. It was a win-win situation.

The events that led to the letter involved a Guernsey cow with twin calves. She had been properly mated to a Guernsey bull, but shortly afterward a lustful Hereford escaped from a neighboring area and got into the act. The color patterns of the calves showed clearly that the twins had different fathers. Blood analysis revealed that the cow carried (among many others) antigen G. The Guernsey bull had antigens S and X_2, while the Hereford bull had R and I′.

The big surprise came with the calves. They had identical blood groups. This could not be explained by their being identical twins, for they were of different sexes to say nothing of having different fathers. Furthermore, *each* twin had antigens from the mother and from *both* sires, G S X_2 R I′. Why should nonidentical twins be identical for these blood groups (and for several others)? How could a calf inherit blood groups from both fathers?

RAY OWEN soon did a differential hemolysis, destroying cells of certain genotypes, and thereby demonstrated that each twin indeed had two kinds of red blood cells. One cell type was G S X_2 and the other was R I′, which made genetic sense. RAY was familiar with the peculiar uterine anatomy of cattle, which facilitates cross-connections between the extra-embryonic blood vessels of the twins (LILLIE 1916). These anastomoses provide a ready opportunity for exchange of blood between the two embryos.

RAY, with his rural background, had long known about "freemartins." These are frequently found when a female calf is born twin to a male. Such a female develops into a sterile, intersex-like adult, totally useless to breeders and dairy farmers. Long before, LILLIE (1916) had demonstrated the union of circulatory systems of twin cattle embryos and postulated that hormones from the male suppressed the normal sexual development of his sister. The blood group admixture showed that more than hormones were exchanged.

The study was soon extended to a large number of twins, and most of the time they were found to share identical blood groups (OWEN 1945). There were no regular proportions of the two types of cells, but whatever the proportion, it was similar in both twins. Thus, the vessels must be broadly connected so that the blood cells of the twins are thoroughly mixed. Are embryonic germ cells exchanged? Possibly yes, but one twin sired 20 progeny yet failed to transmit those antigens that he had gotten from his co-twin. Thus, the mixing of blood cells did not, at least in this case, extend to any mixing of germ cells. RAY's most spectacular example was a set of cattle quintuplets born on a farm in Nebraska (OWEN

FIGURE 1.—RAY OWEN around 1960.

et al. 1946). Four calves were male, one was female, and all had identical blood groups. Each quint had three identifiable kinds of blood cells representing at least three genotypes; very likely there were five.

An immediate practical consequence of this work was that freemartins could be identified as very young calves. If opposite-sexed twins showed mixed blood types, the consequence of fused vessels (which occurred about 90% of the time), the female calf could be predicted to develop into a freemartin. The breeder could sell this one for veal and save the costs of feeding a calf that would turn out to be sterile. For many years the immunogenetics laboratory at Wisconsin offered a valuable freemartin-identifying service to cattle raisers.

Another feature of the blood mixtures was quickly noted. The chimerism persisted far beyond the maximum life of blood cells. Therefore, what had been exchanged between the twins included blood cell precursors, not just the blood cells themselves. In fact, the antigenic phenotype persisted throughout the animals' lives.

The blood admixture challenged a fundamental immunological tenet. Ordinarily, transfusion of blood from one individual to another leads to a specific, often severe transfusion reaction. Yet, somehow, each twin had survived and thrived, despite a massive transfusion of incompatible cells from the co-twin. Why should this embryonic exchange be exempt from the regular rules of blood transfusion?

RAY wrote a longer paper discussing this question and foreseeing the possibility of what was later to be called immune tolerance. Unfortunately, the paper was rejected and only a much shorter one was published (OWEN 1945). It included only an explanation of the exchange, with no discussion of possible immunological implications. Alas, no copy of the first, unpublished paper can be located. It would be a great find for historians.

A few years later, another serendipitous discovery was made, this time on the other side of the Atlantic. The late HUGH DONALD, who did research on animal breeding in Edinburgh, was looking for identical twin calves. A genetically identical twin provides the perfect control for many kinds of experiments. The statistical gain to be gotten from reducing the between-twin variance was well understood, and identical twin calves were eagerly sought by researchers. The problem was, and is, that identical twins are rare in cattle. Also, especially in pure breeds with uniform color, it is often quite difficult to distinguish the two types of twins in newborn calves. In their search, DONALD and his colleagues (1951) had made a rare find: identical quadruplets, identified as identical by exhaustive phenotypic analysis. I am sure the experimenters wished for many more; it would have been a statistical bonanza.

At the 1948 International Genetics Congress in Stockholm, DONALD encountered by chance PETER MEDAWAR, who at the time was working on tissue transplants in mice. MEDAWAR—over a cocktail, it is said—was certain that skin grafting would be an easy, certain way to distinguish between identical and fraternal twins. So he and his colleagues began an extensive grafting experiment in cattle. To their amazement, skin grafts were accepted by almost all the twin pairs, including those of the opposite sex.

On still another continent, F. MACFARLANE BURNET and FRANK FENNER in Australia were developing the concept of self and non-self in antigen recognition (BURNET and FENNER 1949). Their book included a reference to OWEN's work. According to MEDAWAR, he read this, and the solution to the mystery was quickly apparent and soon published (ANDERSON *et al.* 1951; BILLINGHAM *et al.* 1952, 1953). Another version is that the connection with OWEN's work was first noticed by DONALD. Whatever the exact sequence of recognition, the explanation for the graft acceptance was immediately clear, and the new science of immune tolerance was born.

Still a third party was involved at about the same moment, this time in Czechoslovakia. MILAN HAŠEK was a follower of LYSENKO and MICHURIN (KLEIN 1985). Impressed by the graft-hybrid results in plants claimed by LYSENKO, HAŠEK decided that double-yolked eggs and parabiotic twins in chickens and between chickens and ducks would be an elegant way to demonstrate such effects in animals. The vascular connections seemed an excellent avenue for Lamarckian inheritance. He

clearly showed the exchange of blood cells and the failure of antibody production. The paper, published in 1953, was written in Russian and the interpretation was in accord with Soviet orthodoxy. In 1955 HAŠEK met MEDAWAR and BRENT, who told him of their interpretation in terms of immune tolerance. HAŠEK's views changed and he later adopted Mendelism. He became a leading figure, heading a large, productive laboratory in Prague. Thanks to his leadership, Prague became a world center in immunology. This didn't last, however, for under the 1968 Soviet putsch in Czechoslovakia he became *persona non grata.* He was deposed, his laboratory and assistants were taken away, his co-workers were dispersed, and his subsequent personal life was a series of crises. He died in 1984.

Remember that the cattle twin work was done in the dark ages of immunology. It was still believed that antibodies were molded by protein-folding on an antigen template. Shortly after, BURNET and others formulated the clonal selection hypothesis, and the research target changed from antigen to antibody (see EDELMAN 1994). This was a much more fruitful direction, and the field of immunology was poised to explode; the immune tolerance discovery helped light the fuse.

In 1960 the Nobel Prize in Physiology or Medicine was awarded to BURNET and MEDAWAR for the discovery of acquired immunological tolerance. MEDAWAR assigned much of the credit to his colleagues, BRENT and BILLINGHAM. Some have suggested that HAŠEK should have shared the prize. MEDAWAR, however, thought of OWEN, who after all was there first. In a letter to RAY, MEDAWAR said that he should have been included in the award, a statement that does honor to both men. Others—not including RAY—have also noted his absence from the Nobel Prize list and have wondered why. Yet, how much better this is than to have received the award and have people wonder why, as has been suggested in some instances.

MEDAWAR got into the field of transplants while working with severely burned patients during World War II. He realized that skin grafts from other areas of the same person were permanently accepted, while those (homografts) from another person were eventually rejected (although they might persist long enough to be clinically useful). But, significantly, he found that a second homograft from the same donor was rejected very quickly. This, to him, was strong evidence for the immune nature of graft rejection. He went on to study mice and was involved with this work when the cattle twin question arose.

MEDAWAR was a man of many parts, a twentieth century renaissance man. He was an opera buff (as is RAY OWEN). He studied mathematical logic and mastered the symbols needed to read the RUSSELL-WHITEHEAD *Principia.* He did experiments on such diverse topics as allometry and diffusion, and a number of other subjects (small experiments, he called them). He was fascinated by the transformation of Amphioxus from a highly asymmetric larva into a symmetrical adult and published an article on the evolutionary implications.

MEDAWAR's best known work, aside from immunology, is on the evolution of senescence. He wrote two semi-popular essays on the subject (reprinted in MEDAWAR 1957) that have been widely heralded as the beginning of modern evolutionary theories of aging (*e.g.,* STEARNS 1992, p. 200). Noting that post-reproductive (or post-progeny rearing) selection against deleterious mutations is weak at most, he suggested the accumulation of such mutations as one explanation. A second explanation, now called antagonistic pleiotropy, notes that selection can increase mutations that are favorable at early ages even if they are deleterious later. Although many other geneticists had similar ideas, MEDAWAR set them forth explicitly. He used FISHER's (1930) reproductive value as a measure of age-specific selection intensity. This intuitively appealing idea has been largely replaced by other measures due to HAMILTON (1966), which can more readily be interpreted in terms of gene-frequency change or probability of fixation (CHARLESWORTH 1994, pp. 197ff). But MEDAWAR was clearly on the right track. The relative importance of the two processes is still not clear and is being actively researched (ROSE 1991; CHARLESWORTH and HUGHES 1996).

Like his friends JULIAN HUXLEY and J. B. S. HALDANE, MEDAWAR enjoyed popular writing. He was a fluent writer of gracefully worded, easily understood essays, and many have been republished in book form. An example is a series of Sunday evening lectures broadcast by the BBC in 1959 and published under the title *The Future of Man* (MEDAWAR 1959). His breadth of knowledge is impressive (as is the high level of programming of the BBC). As he said, "A human biologist must be a demographer, geneticist, anthropologist, historian, psychologist and sociologist all in one;" he came close. His mastery of English prose shows on every page. The book led to one minor disagreement. This was with H. J. MULLER, who thought that MEDAWAR overemphasized the importance of overdominant loci for quantitative traits and was therefore too pessimistic about the effectiveness of selection.

Late in life, MEDAWAR suffered a stroke that slowed his physical activity, but not his restless, wide-ranging mind. He continued to read, work (but not with his hands), and dictate essays and books. He published a charming autobiography with the intriguing title, *Memoir of a Thinking Radish* (MEDAWAR 1986). Death came in 1987 at age 72.

RAY OWEN was born the same year as MEDAWAR (1915) and grew up on a Wisconsin dairy farm. For eight grades, he attended a two-room school. Then and through high school, he did his farm chores each day before and after classes. Following graduation from Carroll College, he began graduate studies at Wisconsin with L. J. COLE and worked mainly with birds. His thesis

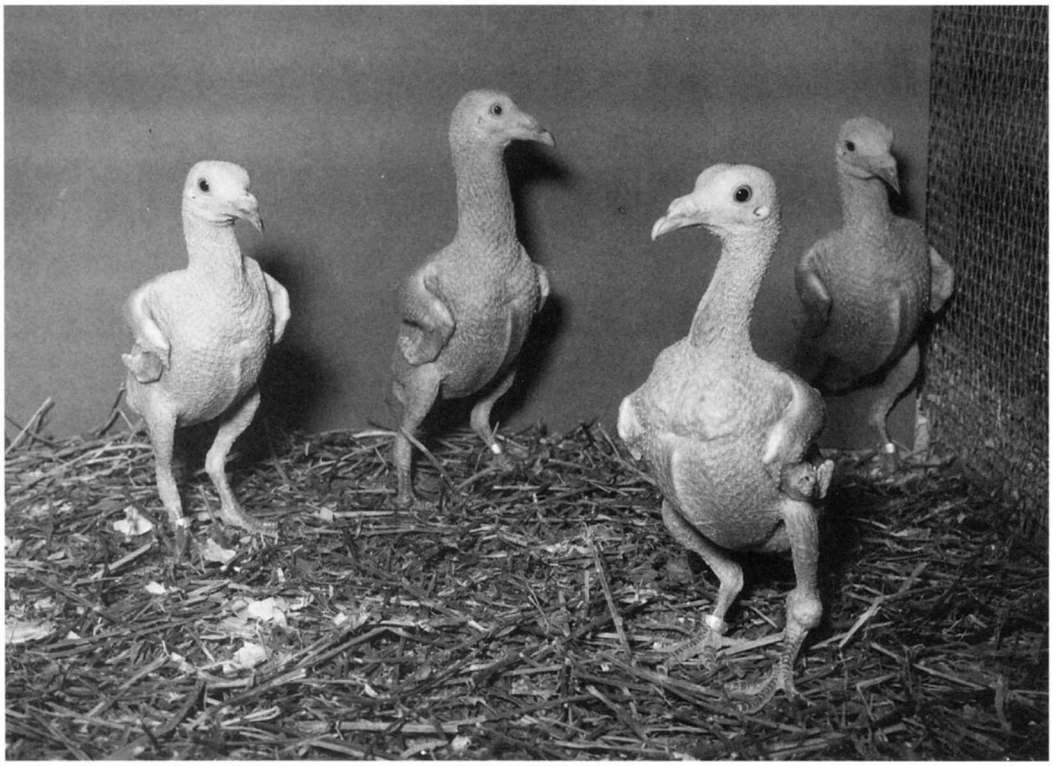

FIGURE 2.—Naked pigeons. These four had 13 normal sibs from a mating between two heterozygous parents.

was on the sterility of species hybrids. The observation of germ-cell migration alerted him to the possibility of such events as were later observed in the twin calves.

One of RAY's early papers—a favorite among his friends—describes "naked" pigeons (COLE and OWEN 1944). These birds, because of a recessive mutant gene, are completely featherless (Figure 2). The paper, largely written by RAY, was published in the *Journal of Heredity*. In those days science was less competitive and publications less crowded, and the editor, R. C. COOK, encouraged humorous, informal, clever writing (and contributed some himself). Here are some choice passages; those who know RAY will recognize the style. "Pigeon courtship, with its strutting, cooing and puffing out of feathers is an interesting performance. When there are no feathers to puff or to clothe the performer, it becomes a ludicrously macabre travesty. . . Although their wings are almost useless organs, these birds seem unable to learn to regard them as such. Placed on a table, they will hopefully take off into space, beating their wings vigorously, as though confident of a controlled landing which, however, ends in a 'crash'. . . They are also active and aggressive lovers. Inadequate attire produces no inferiority complex in them; they strut and coo, puff and bow as if arrayed in the finest of raiment." Alas, matings required artificial insemination and the fertility was low. Keeping the mutant gene by mating heterozygotes proved too laborious, and the strain was lost. "The perpetuation of the strain is so tedious that it will be a long time before the housewife can buy her squabs gene-plucked."

RAY's immunogenetic work was done as a postdoctoral fellow. In 1947 he left Wisconsin for Caltech. The venue changed from cattle barns to rodent labs. Although the emphasis was always on immunogenetics, the organisms were strikingly varied. He and his students studied, in addition to rats and mice, viruses, ciliates, goldfish, birds, and humans. Always a popular teacher, he devoted considerable time to it. Along with ADRIAN SRB, he wrote a pathbreaking textbook (SRB and OWEN 1952) that started a trend in presenting genetics as an active, evolving subject. It quickly became a best seller.

RAY's later record shows a diminished number of scientific papers with his name attached, and there is a reason. In the 1960s, he decided no longer to permit his name to appear on papers by his students when they had done most of the laboratory work. But he continued to suggest problems and to offer assistance and guidance. His helpfulness to students, his and others', is a Caltech legend. RAY is never too busy to help with a problem, be it scientific or personal. Nor is he too busy to accept a difficult administrative task, and this became a large part of his life. At Caltech he has become the students' friend and advocate, and in many ways contributed to the conscience of the institution.

Having recently passed his eightieth birthday anniversary, RAY has been the recipient of well-justified praise by former Caltech students and scientific colleagues. In the summer of 1996 a symposium in his honor was held at the University of Wisconsin. It was an exciting event, marred only by the death a few days earlier of GEORGE SNELL, another pioneering transplant geneticist. The symposium was an intellectual feast. The latest theories

and observations were on display. The contrast between what was known in 1946 and the state of the science in 1996 is amazing. It seems especially so to those, like me, who have viewed the subject with continued interest, but always from the outside. What a difference a half-century makes!

I should like to thank RAY OWEN for helping me with a number of historical details. As a consequence there are far fewer errors than there would otherwise have been.

LITERATURE CITED

ANDERSON, D., R. E. BILLINGHAM, G. H. LAMPKIN and P. B. MEDAWAR, 1951 The use of skin grafting to distinguish between monozygotic and dizygotic twins in cattle. Heredity **5:** 379–397.

BILLINGHAM, R. E., G. H. LAMPKIN, P. B. MEDAWAR and H. L. WILLIAMS, 1952 Tolerance to homografts, twin diagnosis, and the freemartin condition in cattle. Heredity **6:** 201–212.

BILLINGHAM, R. E., L. BRENT and P. B. MEDAWAR, 1953 Actively acquired tolerance of foreign cells. Nature **172:** 603–606.

BURNET, F. M., and F. FENNER, 1949 *The Production of Antibodies.* Macmillan, London.

CHARLESWORTH, B., 1994 *Evolution in Age-Structured Populations,* 2nd ed. Cambridge University Press, Cambridge.

CHARLESWORTH, B., and K. A. HUGHES, 1996 Age-specific inbreeding depression and components of genetic variance in relation to the evolution of senescence. Proc. Natl. Acad. Sci. USA **93:** 6140–6145.

COLE, L. J., and R. D. OWEN, 1944 Naked pigeons. J. Hered. **35:** 1–7.

DONALD, H. P., W. S. BIGGAR and D. M. LOGAN, 1951 Monozygotic bovine quadruplets. Heredity **5:** 135–142.

EDELMAN, G. M., 1994 The evolution of somatic selection: the antibody tale. Genetics **138:** 975–981.

FISHER, R. A., 1930 *The Genetical Theory of Natural Selection.* Clarendon Press, Oxford.

HAMILTON, W. D., 1966 The moulding of senescence by natural selection. J. Theor. Biol. **12:** 12–45.

KLEIN, J., 1985 In memoriam. Immunogenetics **21:** 105–108.

LILLIE, F. R., 1916 The theory of the free-martin. Science **43:** 611–613.

MEDAWAR, P. B., 1957 *The Uniqueness of the Individual.* Basic Books, New York.

MEDAWAR, P. B., 1959 *The Future of Man.* Methuen and Co., London.

MEDAWAR, P. B., 1986 *Memoir of a Thinking Radish.* Oxford University Press, Oxford.

OWEN, R. D., 1945 Immunogenetic consequences of vascular anastomoses between bovine twins. Science **102:** 400–401.

OWEN, R. D., 1989 M. R. IRWIN and the beginnings of immunogenetics. Genetics **123:** 1–4.

OWEN, R. D., H. P. DAVIS and R. F. MORGAN, 1946 Quintuplet calves and erythrocyte mosaicism. J. Hered. **37:** 291–297.

ROSE, M., 1991 *The Evolutionary Biology of Aging.* Oxford University Press, Oxford.

STEARNS, S. C., 1992 *The Evolution of Life Histories.* Oxford University Press, Oxford.

SRB, A. M, and R. D. OWEN, 1952 *General Genetics.* W. H. Freeman, San Francisco.