CITATIONS MOST FOWL

by Bernard Dixon

There are respectable reasons why certain research papers are cited extraordinarily often in the scientific literature. "Lowry, Rosebrough, Farr, and Randall, 1951"—the paramount example—still seems to crop up at least once in every issue of every biochemistry journal. At latest count, this report of a novel method of measuring soluble protein has been cited 187,652 times since Oliver Lowry and his colleagues at Washington University (St. Louis, MO) first reported their neat and sensitive technique in the *Journal of Biological Chemistry* (**193:**265, 1951). Other high-flying reports include early descriptions of Southern blotting, DNA sequencing, and the production of monoclonal antibodies. Since their publication in 1975, 1977, and 1975, these papers have attracted 16,382, 10,718, and 5,995 citations, respectively.

Critics of citation analysis argue that figures of this sort give unwarranted prominence to experimental procedures, as compared with Nobel prize-winning adventures of the mind. They have a point—though not, I believe, one of more than marginal significance. Should any of the above papers really be derided as "mere" methods? Is it not more reasonable to see each of them, in its own way, as a major thread in the historic fabric of science?

Whatever the resolution of that debate, let me highlight my own *bête noir* of the citation business. This is the type of reference, usually brandished in advocacy, that acquires a distinctive character over the years simply because it is rarely accompanied by citations of supporting work. To be specific: At least once a year over the past decade, I seem to have read a paper or article discussing the idea of preventing chickens from being colonised by Salmonella, by inoculating them with a protective bacterial flora. The authors enthuse over the principle of "competitive exclusion," and highlight the likely benefits of so-called "probiotics" in humans, too. Yet their one solid piece of evidence has usually been a single paper entitled "New aspects of Salmonella infection in broiler production," published in 1973 by E. Nurmi and M. Rantela (*Nature* **241:**210, 1973).

This was certainly an intriguing paper. Nurmi and Rantela indicated that dosing newly hatched chicks with a suspension of intestinal bacteria from adult chickens could increase their resistance to later invasion by disease-causing Salmonella. The work was competently performed, and the results sufficiently positive to be encouraging. But "Nurmi and Rantela, 1973" was a very limited study, which underlined the need for more detailed research—not least in determining the permanence of the phenomenon, discovering its underlying nature, and strengthening the effect by using defined inocula and protocols. Instead of developing these leads, however, later authors continued to hang most of their case on Nurmi and Rantela. They in turn made haste slowly and published no further papers in high-visibility journals such as *Nature*.

Now let me venture a prediction—that the boom in citations reflecting this peculiar pattern of publication is about to end. Firstly, Esko Nurmi and his colleagues at the National Veterinary Institute in Helsinki have now made really tangible progress. Secondly, other groups have joined in to establish competitive exclusion as a sound basis for protecting flocks (and thus humans) against Salmonella infection.

The most impressive new evidence is from Sweden, where Nurmi and co-workers gave cultured caecal material to 2.86 million broiler fowls. Most of the birds were from units where previous flocks had suffered Salmonella infection. Yet only one of the 144 treated flocks became infected, compared with 87 of 144 untreated flocks. There were no adverse effects on the chickens' health or performance.

A Dutch study involving eight million broilers has produced similar, though less significant, results. And in Britain, Geoffrey Mead and others working for the AFRC Institute of Food Research (Bristol) have reported that competitive exclusion prevented reinfection in 20 of 22 trials on 250,000 chickens, ducks, and turkeys when used after antibiotic therapy.

Progress is being made, too, in characterising the protective organisms; understanding the nature of their action; and, most recently, developing improved procedures for field application. The Farmos Group in Turku, Finland, has launched a mixed culture, "Broilact," for farm use. Recent collaborative work between Nurmi, Mead and co-workers (*The Veterinary Record* **126:**510, 1990) shows that Broilact is highly effective in preventing Salmonella colonisation when administered to newly-hatched chicks as droplets from a hand-held spray. This technique, which ensures that the protective flora spreads evenly among the birds, can be used immediately after hatching. These are two advantages over the inoculation of drinking water, as used in most previous experiments. The new method could easily be automated.

Scrutiny of normal intestinal flora for individual protective strains is far from concluded. On the one hand, anaerobic bacteria are thought to play a major role, as illustrated by reports that certain Clostridia confer protection. On the other hand, Paul Barrow and colleagues in the Houghton laboratory of the AFRC Institute for Animal Health have protected poultry against very high doses of *Salmonella typhimurium* by using the avirulent, rough F98 strain of this same organism (*Epidemiology and Infection* **104:**427, 1990). The inhibition was *not* a result of immunity or bacteriophage activity. Although the chicks resisted only five of nine *S. typhimurium* strains, this seems to be a consequence of the poor colonising ability of F98. A better coloniser may well provide a wider spectrum of defence.

Competitive exclusion seems set to become a fashionable pursuit—certainly in animal husbandry, if not in human medicine too. And this means that "Nurmi and Rantela, 1973"—unlike Lowry *et al*, 1951—has been superceded into oblivion.