Little Fleas and Lesser Fleas
The Seventeenth Marjory Stephenson Memorial Lecture

By PETER WILDY
Department of Pathology, University of Cambridge, Tennis Court Road, Cambridge CB2 5JW, UK

(Delivered at the 103rd Ordinary Meeting of the Society for General Microbiology on 2 April 1985)

Ruder heads stand amazed at these prodigious pieces of nature, whales, elephants, dromedaries, and camels; these, I confess, are the colossus and majestick pieces of her hand; but in these narrow engines there is more curious mathematicks; and the civility of these little citizens more easily sets forth the wisdom of their Maker.

Sir Thomas Browne, 1642

This effulgent piece of prose by the erudite, and erratic, Thomas Browne was chosen by Marjory Stephenson as a banner to head the third edition of her famous book *Bacterial Metabolism* (Stephenson, 1949). Indeed the distinction of the banner matches the distinction of the author whom we remember periodically with this eponymous lecture. I feel greatly honoured, and undeservedly so, to be allowed to stand up and pretend the sort of worthiness that a Marjory Stephenson Lecturer should possess.

When we are young, we have our gods and goddesses - a satisfactory state of affairs that unfortunately dissolves with ageing. Marjory Stephenson was one of my Goddesses (I refrain from mentioning the others) and it has always been a sadness that I never to my knowledge met her. Apart from her distinction she seems by all accounts to have been such a great human being and she was such a good gardener. She pioneered a very considerable chunk of biology. The prefaces of the three editions of her famous book bear testimony to some thirty years of microbiological progress (before, as a matter of fact, microbiology had been invented). Thus we see each edition embracing the previous decade. The first edition was prefaced (in 1929) by a confession of the great difficulty in presenting a coherent picture of microbiological progress. There was allusion to examination of the 'contents of the dustbin' from which deductions might be made as to the events occurring 'above stairs'. She noted concern about the place of bacteria in evolution and the difficulty in assessing how far the forms we understand resemble primitive bacterial types, or whether like modern animals and plants they are the successful competitors of the ages. Bacteria, perhaps, might be envisioned as 'biochemical experimenters'; few of us today would seriously disagree with that. She understood very well that the extraordinarily rapid multiplication rate of bacteria would allow the study of genetic variation on an unprecedented scale.

Ten years later we find an enthusiastic preface to the second edition. Microbiology in 1939, though a young science, has begun to gain confidence. On bacterial growth Stephenson remarks: 'happily this subject now attracts mathematicians and statisticians less than formerly but has passed into the hands of biochemists interested in the problems of nutrition. This has led to results of both theoretical and practical importance and has revealed *inter alia* that the complex and peculiar media employed by bacteriologists in the cultivation of "difficult" pathogens are rendered necessary owing to the inability of many parasitic organisms to synthesize for themselves certain molecules essential for growth.' This particular concept correlates with the views put forward by Fildes (1934) that parasitism is caused by loss of enzymes necessary to synthesize cell material. The first stage from autotrophy to heterotrophy forces free-living
organisms to depend on others; enzyme losses result in further degrees of dependence and in the extreme compel a state of obligate parasitism. The serial nature of this concept of evolving parasitism is for some reason aesthetically attractive. We notice it again in the contemporaneous hypothesis on the origins of viruses (Green, 1935; Laidlaw, 1938). Also belonging with this era we find Theobald Smith's classical commentary on the relation of parasitism and disease (Smith, 1934).

Ten years later again the preface to the third edition of *Bacterial Metabolism* marks the beginning of the great microbiological acceleration. Isotopic labelling methods had been developed and had vastly improved the scope of experimentation. Useful work had developed in the fields of microbial genetics, nucleic acid metabolism, adaptive enzyme formation, bacterial metabolism and effective chemotherapy. One might also add the work of the Cold Spring Harbor school which, during this decade, was to prove seminal in virology.

It is a pity that Majory Stephenson did not live to see the big biological explosion that has happened in the last 35 years. But she and the school she founded accomplished much distinguished work in this preparatory period. In asking what has happened in the past 35 years the answer is nearly everything. We understand the composition, structure and mode of working of genetic material and information transfer, man has trodden on the moon, the technology of handling large molecules has become simple, we can make monoclonal antibodies, there is a national health service (just), not only do we understand how large molecules work but we can manipulate genetic material more or less as we want and generate 'constructs', there are women in the House of Lords, no-one can do arithmetic any more but we have excellent computers which faithfully serve us provided that we punch in the correct information. We can easily determine sequences in large molecules. Our excellent railway system is practically dismantled, everyone seems to split their infinitives, we talk of 'scenarios', and nobody can spell. I shall be accused of flippancy, perhaps justly, but the above catalogue draws attention to the fact that those of my age are living in a quite different world from that in which we grew up; perhaps it will help others to understand our curious antique ways.

Germane to the subject of this essay there have been two particularly important conceptual innovations. They are first that parasites which we always believed transmitted horizontally can now in some instances be transmitted vertically in germ-plasm: for instance the endogenous retroviruses (Payne & Chubb, 1968). The second is that genes which we confidently thought of as being transmitted vertically from parent to progeny are now well-known to be transmissible horizontally by a variety of agents. While transmission of microbial genes into their hosts was of course accepted, mostly their subsequent function was regarded as being wholly concerned with the microbe and not with the host. There were two observations which obtruded invitingly against this bland generality, lysogeny (Bordet, 1925) and pneumococcal transformation (Griffith, 1928). These curious findings became fascinating talking points in biological circles but orthodoxy admitted only vertically transmitted genes and horizontally transmitted viruses, and concepts of evolution were affected accordingly. However, Schimper was arguing as early as 1883 that plasmids arose from endoparasites (Cavalier-Smith, 1981) and Margulis (1970, 1981) makes a strong case for the endosymbiont theory of the origin of organelles like plastids, mitochondria and cilia. Such views nowadays command general support.

So naturalists observe a flea
Has smaller fleas that on him prey;
And these have smaller still to bite 'em;
And so proceed *ad infinitum*

Swift, Poetry, a Rhapsody

There are many versions of Dean Swift's little verse; some may be more sonorous, better scanned, more aptly rhymed or mathematically more satisfactory. I prefer the original. The main theme that I now wish to embrace is parasitism and the definition I shall use is the very broadest. A parasite is an entity that lives at the expense of a larger entity, its host. The entities may be organisms or other biologically organized systems. The parasite may or may not pay something back to the host; if it does it is a symbiont.
Perhaps we should deviate a moment to consider what attributes a successful parasite should possess. Essentially, if the parasite were to affect the host adversely and could not evade the host's defences, the one option for its survival is to clear out. It must have plans for an escape route and if it is an obligate parasite it must find a new host. The important consequence of this is that the species survival of this parasite depends largely upon the concentration of fresh susceptible hosts. For instance, measles virus infection happens to cause sufficient aggravation to the human host that one perceives clinical symptoms literally in every infection. There are virtually no secondarily infected cases. If we compare this virus infection with chicken-pox we find that the latter gives rise to a strong immunity. Nevertheless 50 years later the patient may succumb to shingles (herpes zoster) caused by the original virus infection. In the meantime the virus has remained alive but latent in the nervous tissue of the particular patient. Since shingles is infectious the parasite has apparently cunningly arranged to be available (very infectiously) for the attention of grandchildren or even great grandchildren. It is easy to understand why measles virus requires a minimum community of $3 \times 10^5$ hosts for its survival while varicella zoster virus requires fewer than $10^3$ (Hope-Simpson, 1965). Varicella zoster virus is the more efficient parasite; very likely herpes simplex is even more efficient. Really stupid parasites cause disease; they possess damage factors which are duly taken note of by GMAG, ACDP and such committees. Parasites that are insufficiently clever to remain in a host for a reasonable tenure may take advantage of various idiosyncrasies of the host to find a new one. Dawkins (1982) takes us into the realms of various behavioural advantages. For example the transmission of a respiratory parasite is probably greatly aided by the host's coughing and sneezing reflexes. Those afflicted with venereal infection are aiding their parasites through (a) their promiscuous habits and (b) their libido; Dawkins's question is: can the parasites encourage those attributes of the host that indirectly benefit them?

In the vertebrate host where there is so well-developed an immune response, successful parasites have evolved various means of evading rejection (Mims, 1982). We have recently been impressed with the aptness of herpes simplex virus at evading both humoral and cellular immunity (Simmons & Nash, 1984). In an experimental mouse model it is clear that intradermally inoculated virus becomes inaccessible to the immune apparatus within a few hours; it multiplies and goes to roost in the local sensory ganglion. Within 3 days it has spread via axons to other parts of the same dermatome and there is a period of only 6 h during which very high concentrations of a neutralizing monoclonal antibody may actually prevent a zosteriform eruption occurring. Once the virus is established in the peripheral nervous system it appears that specific protective lymphocytes are without effect. Moreover the likely explanation is that the virus antigen - readily demonstrable in the epidermis by labelled antibody - is quite invisible to the immune system. The longer-term outcome of a herpes infection in mice or man is of course the establishment of latency in the local sensory ganglion, a fact that may be verified by simply explanting ganglia intact. After incubation for a few days the virus (which cannot be demonstrated in freshly removed ganglia) reactivates and may be detected in both mouse and man as infective virus (Stevens & Cook, 1971; Baringer & Swoveland, 1973). There is good evidence that the latent virus occupies the sensory neurone and that there is no expression of virus antigens until virus reactivation (McLennan & Darby, 1980). Until lately, we had no information about the state of the virus genome during latency. Recent work shows that the DNA is 'endless', unlike the linear molecule found in the virion (Rock & Fraser, 1983). This endless form has been found in both mouse and man (S. Efstathiou, personal communication). In natural herpes, virus reactivates from time to time and must pass centrifugally from the ganglionic neurones to the skin, where it may establish a recurrent infection, a recrudescent lesion, and thus be shed. Throughout its sojourn in the host the virus evidently occupies a privileged situation; but now and again it will cut and run.

The above story illustrates very well the advantages of a parasite maintaining a low profile. The final path towards a symbiont is illustrated most easily by the green animals Hydra viridis and Paramecium bursaria, both of which harbour Chlorella endosymbionts. There seems to be some evidence of strain specificity between the chlorella and either animal. In the light both animals can make do with scarce nutrients better when parasitized by chlorellae. Sexuality in
this paramecium evidently requires the presence of chlorellae. In the light each partnership maintains roughly constant proportions of endosymbiont. Under appropriate conditions either host or endosymbiont may be cultivated apart from the other but symbiosis is more readily re-established with the symbiont strains of chlorella than with other strains. If *Hydra viridis* is exposed to intense white light, it will expel its intracellular chlorella (for references see Margulis, 1981). Some very interesting results relating to these textbook symbionts were recently reported by Van Etten et al. (1982). These authors were able to maintain the animals in appropriate conditions after ridding them of chlorellae but they were unable to cultivate the algae. Indeed though these little cells photosynthesized very well immediately upon separation from the animals they became quite inactive 18 h later. It became plain that explantation of the chlorellae induces a virus in them first seen at 8 h. This multiplies and lyses the chlorella (reminiscent of herpes reactivation). It turns out that such viruses were generally reactivated in this way from their endosymbiont algae regardless of geographical source. The viruses were all similar with 185 nm diameter particles and double-stranded DNA (G + C ratio ≈ 50%). Four types of virus were distinguished using restriction analysis and serology. Though not rigorously proven, it seems likely that the algae carry a lysogenic virus. The story is intriguing; it is evident that the animal host must somehow help in the maintenance of the lysogenic state and one is tempted to speculate that the algal surface must carry some virus-coded marker.

The stable association that a parasite has with its host must be the result of a well-timed biological balance and it should thus be particularly rewarding to examine examples like the above where ‘third parties’ are involved and may play a role in controlling the balance.

The matter of third parties is of interest and importance in parasitic economy. Probably the most frequent alliances involve parasitic bacteria and their hosts and phages, plasmids or other transmissible genetic systems. For instance *Corynebacterium diptheriae* would be an innocuous surface parasite were it not for the fact that the β-phage lysogenizes it and the conversion protein happens to be the exotoxin (Freeman, 1951). The third party – the lysogenic bacteriophage – spoils what otherwise might be a happy and longlasting association. It can scarcely be imagined that the third party makes for comfortable living for any of this triad. If we move to the gut of any vertebrate we find a veritable ‘zoo’ of bacteria and viruses – occasionally of protozoa too – all living in some sort of balance with the host. The practical issue here has been the transmission of antibiotic resistance genes by plasmids and other entities; resistance genes are transmitted from plasmid to plasmid, from microbe to microbe, from gut to gut and from pigs to man. Toxin genes of enteric pathogens are also distributed in this way. Recently, a toxin active against tissue culture cells and important in oedema disease in piglets has been found to be the product of a bacteriophage gene. The phage then transmits virulence to *Escherichia coli*, and disease results (Williams Smith et al., 1983).

The few examples we have considered represent a tremendous diversity of organisms and relationships. It would be difficult and unrewarding to classify them. However, there is a very important distinction to be made in the nature of all parasitic relationships; most associations are in effect complementations, others are genetical. It will be obvious that the parasitism described in the green hydra is of the first kind even though the association is close – the alga is intracellular. On the other hand the phages, plasmids and resistance factors are parasites of the genetic kind.

Just as reproduction insures the perpetuation of existing species, the author believes that Symbionticism insures the origin of new species.

I. E. Wallin, 1927

Lynn Margulis uses this quotation to introduce the Endosymbiont Theory of Evolution (Margulis, 1981). Essentially this depends upon the distinction between prokaryotes and eukaryotes and the evolution of obligate symbiosis. The theory nowadays relates mainly to the evolution of organelles in eukaryote cells in particular plastids, mitochondria and cilia. It is easy to see how, for instance, chloroplasts may have developed from endosymbiont photosynthesizing prokaryotes and to imagine how time might allow the moulding of such entities toward the
organelles we know today. In a superficial way the green animals referred to earlier, or the lichens, resemble the kind of photosynthesizing symbiont that could be considered as an early precursor. In these particular instances of course the parasite is itself an alga. There is no dearth of circumstantial evidence that chloroplasts were very probably derived from photosynthesizing cyanobacteria. They have their own genetic system which is somewhat like that of a prokaryote, specific DNA, 70S ribosomes, general prokaryotic organization, and they can be removed, at least from *Euglena gracilis*, by treatment with a large number of different antibiotics (Ebringer, 1972); the organism then becomes colourless and may be grown on appropriate nutrients. Finally, there is greater homology between the 16S rRNAs of chloroplasts and cyanobacteria than between those of chloroplasts and the cytoplasm in which they reside. Though it is easy to see how symbionts could have synthesized fresh organisms, and there is evidence to support this, I have found difficulty in understanding what manner of selective pressure could mould the new chloroplast-maintaining entity. Margulis (1981) points out that there is likely to be a strong synergistic element. A strong symbiotic association is better than the sum of its parts. Nevertheless this certainly improves the survival advantage of both partners; one must suppose that progressive loss of unnecessary attributes makes the partnership yet more efficient. Another difficulty has been to understand how the symbiosis develops an 'intelligent' interaction between the partners. I find myself quite startled by the finding, mentioned above, of a lysogenic virus in the algal partner of the green hydra; it offers the possibility that the 'third party' may well be the genetic engineer needed to turn a complementation symbiosis into a genetically harnessed duo.

When we come to examine the genetical symbioses we find no difficulty in appreciating their very obvious potential as latter-day information vendors. To list some of the transposable elements that have been described, there are plasmids, insertion sequences, viruses, phages, transposons, replicons and episomes. They may carry genetic sequences from cell to cell when they may be expressed. We have already referred to their participation in vertebrate guts. These fascinating genetical tearabouts clearly distribute information between bacteria, but what about other species? One plasmid that appears to be introduced as an intelligible message from prokaryote to eukaryote is the Ti plasmid carried by *Agrobacterium tumefaciens*, which invades and is understood by dicotyledonous plants, resulting in the formation of crown gall tumours. Put briefly, the travelling sequences require some factor or entrepreneur to ensure their safe delivery.

Among vertebrates we are blessed or cursed by the retroviruses, which are able occasionally to pick up chromosomal genes when inserting and removing viral sequences from host-cell DNA. Such genes are transcribed to RNA and may be carried as part of the viral genome. RNA is transcribed to DNA by the enzyme reverse transcriptase, the discovery of which must be the most important biological finding of this century (Temin & Mitzutani, 1970; Baltimore, 1970). When the virus genome inserts into a host-cell chromosome it may promote neighbouring genes such as cellular oncogenes. Furthermore it may introduce and switch on similar genes - virus oncogenes - after insertion into the host-cell chromosome. If bacteria are 'biochemical experimenters' retroviruses are genetic engineers.

Some of the retroviruses, called endogenous viruses, remain buried as part of the host-cell DNA and can only be reactivated and recovered as virus sequences with difficulty, for instance by reactivation with BUdR. However, when that is done the induced virions are capable of analysis by hybridization experiments. One of the electrifying results of the last decade has been the discovery that sequences transferred long ago between species have endured by vertical transmission for long periods. For example, Benveniste & Todaro (1974) reported that the DNA of many primates contains sequences related to an endogenous cat virus (RD-114); it was concluded that a virus originally of primates must have infected a feline and become stabilized 10⁶ or 10⁷ years ago. Other examples have related mice to woolly monkeys and gibbons, rats and cats; pigs and rodents (for references see Reanney, 1976).

Oncogenes are normal evolutionarily conserved sequences in vertebrate chromosomal DNA. They are perceived in two forms, as cellular oncogenes, which like other eukaryote genes may have introns, and as virus oncogenes in the viral genome. As with other retrovirus genes this
latter form is not spliced and is imprinted alternately in DNA and RNA. The oncogenes are involved with various cancers and have been christened with indicative three-letter names derived usually from the tumour of origin; thus we have src, fes, sis, etc. The prefixes c- and v- are constructively used to denote cellular or virus. The gene functions are in some cases known, thus c-sis specifies a polypeptide in platelet-derived growth factor, v-erb specifies the reception for epidermal growth factor, v-src and v-abl have associated tyrosine protein kinase activity; this enzyme is probably important in cell transformation. Both v-ras and c-ras produce a protein with high affinity for guanosine triphosphate. The switching on of transcription may be affected by a number of factors but the long terminal repeats in the retrovirus genome have a powerful role both as promoters and enhancers (Gallo & Wong-Staal, 1984; Hunter, 1984; Vines, 1985). How do oncogenes rate as parasites?

Though somewhat arbitrarily, we generally choose to regard the c-oncogenes as part of the vertebrate host since they have the general characters of host genes, including introns. But when the RNA transcripts are transcribed back into DNA there is no splicing back of intron so that we now have a new object which can be thought of as a molecular parasite of either the vertebrate or the virus host. So we have come in a roundabout way to the position Dawkins (1976, 1982) has chosen to adopt in his treatment of the ‘selfish gene’ and its extension. We see not only ourselves but the retroviruses as mere survival machines for the benefit of gene patterns.

Finally we come to consider the ultimate parasite. In the sense of Theobald Smith (1934) this would be versatile, would comprise the smallest possible genetical apparatus (testifying its extreme efficiency) and the least possible non-genetic matter, and it would require a generous or at least a sufficiently provident host. It would take care not to offend the host and would apparently love the host. Mind you, it might not be above being deceitful, as Dawkins has pointed out. Perhaps a satellite virus such as tobacco necrosis satellite comes close to perfection. It consists of one smallish molecule of RNA wrapped up in a shell made of a single kind of protein. It apparently does little harm, however it requires a host virus, itself a parasite of another organism, and the two must enter the same cell – the association is specific. This is not a versatile parasite. How does herpes rate as a parasite? Not bad. The virus is immensely successful, colonizing almost 80% of its available hosts. It has evolved means of dodging host immunity and makes the maximum use of time by its programme of latency and recrudescence. What of the chloroplast? Assuming this to be the ultimate extension of a symbiotic relationship the position seems moderately satisfactory. But the chloroplast must work for its living. We finally come back to the oncogene or ‘just’ the selfish gene as concluded by Orgel & Crick (1980). However, for maximum success the selfishness must not take the form of aggravation, indeed the gene must appear like Jeeves – rather self effacing.

That’s a valiant flea that dare eat his breakfast on the lip of a lion.

William Shakespeare

King Henry V, iii, 7

REFERENCES


