BACTERIOPHAGE RESEARCH

SECOND ROYAUMONT COLLOQUIUM

IN the summer of 1952, André Lwoff, of the Institut Pasteur, organized an International Bacteriophage Colloquium at the Abbaye de Royaumont near Paris, the first reunion of European and American phage workers since the renaissance of the early 1940's of interest in bacterial virus. That meeting was held at a crucial time in phage research; for it had only just been discovered then that the tail of the phage is the organ of attachment, that the deoxyribonucleic acid of the phage is the carrier of its hereditary information, that genetic recombination of the phage can be understood in terms of population genetics, that the prophage of lysogenic bacteria is located on the bacterial chromosome, and that genetic recombination in bacteria is a unidirectional process involving donor and receiver cells. It was, therefore, fortunate that an international group happened to meet to appreciate these novel findings, which permitted old questions to be reformulated at a higher level of sophistication.

An unfortunate change has occurred since that Colloquium, when it was still the rule that all first-rate phage workers were always aware of everything important in their entire field. In the past six years, phage research has experienced such a remarkable development that there remain few, if any, persons who still retain complete mastery of every aspect of knowledge of bacterial viruses. Hence, it was appropriate that a Second International Phage Colloquium should be held at Royaumont during July 28–August 2, attended by sixty workers from Australia, Belgium, Denmark, France, Germany, Great Britain, Italy, Japan, Hungary, Switzerland and the United States. No papers were read (nor lantern slides projected) at this meeting, nor will there be any publication. Instead, each session was devoted to the discussion of a given topic under the leadership of a chairman, after an introductory talk on the subject by a *rapporteur*, according to the following programme:

TOPIC	CHAIRMAN	RAPPORTEUR
Anatomy of the Phage Particle	E. Kellenberger (Switzerland)	S. Brenner (Great Britain)
Metabolism of Infected Bacteria	R. M. Herrkott (United States)	E. Volkin (United States)
Synthesis of Phage Constituents	O. Maaløe (Denmark)	G. Streisinger (United States)
Kinetics of Recombination	A. H. Doermann (United States)	C. Bresch (Germany)
The Elementary Recombination Act	C. Levinthal (United States)	R. Edgar (United States)
Genetic Fine Structure	G. S. Stent (United States)	S. Benzer (United States)
Replication	F. H. C. Crick (Great Britain)	F. W. Stahl (United States)
Prophage and Chromosome, Transduction	E. S. Anderson (Great Britain)	J. Lederberg (United States)
Phage Conversion	B. A. D. Stocker (Great Britain)	N. Groman (United States)
Bacteriocines	G. Ivanovics (Hungary)	P. Frédéricq (Belgium)

As is evident from this programme, the discussions ranged over a wide field, and to attempt a synthesis of their outcome would be a formidable task indeed. Hence only a rather fragmentary selection of a few of the more important developments to emerge at these proceedings can be mentioned here.

Refined electron, optical and chemical studies reported by S. Brenner have shown that the tail of

the bacteriophage consists of three proteinaceous parts: an outermost, cylindrical sheath, enclosing a hollow cylindrical core to which tail-fibres are attached at the distal end. The tail fibres seem to represent the real adsorption organs of the phage, since isolated tail fibres are fixed specifically to bacteria and contain almost all the serum-blocking power of the phage, in contrast to isolated sheaths and cores which possess neither of these properties. The phage, it seems, has a spring; for the sheath is a contractile organ which shortens and thickens when the tail of the phage makes contact with the host cell surface. The sheath, furthermore, is the site of action of adsorption co-factors and inhibitors, which affect the adsorbability of the phage by their influence on a reversible interaction between tail fibres and sheath. The following picture of the infection process may now be inferred from these facts: after phage and bacterium have collided, the phage tail-fibres flail about until they have made a stereospecific fit with the bacterial receptor sites and have anchored the particle firmly to the host surface. The sheath then contracts and pushes the core through cell wall and membrane, so that the viral deoxyribonucleic acid may pass from the head of the phage through the hole in the core into the interior of the bacterium.

Once in the interior of the infected bacterium, the viral deoxyribonucleic acid fulfils two tasks. It replicates itself several hundred-fold to produce the hereditary substance for the progeny particles and it induces the synthesis of viral protein. Parts of both of these processes can be seen directly in some striking electron micrographs of phage-infected bacteria, obtained by E. Kellenberger by means of novel staining and ultra-thin sectioning techniques developed in his laboratory. These pictures show how, in the course of intracellular phage growth, the bacterial 'nucleoids' disintegrate to give way to a pool of viral deoxyribonucleic acid, and how this pool at later stages (but prior to its encapsulation into morphologically intact, infective virus particles) crystallizes into hexagonal, pre-phage structures, which break up into free deoxyribonucleic acid and protein 'doughnuts' if the cell is lysed prematurely. The phage precursor deoxyribonucleic acid thus exists in at least two different states before its maturation, a fact which must be reckoned with if biochemical and genetic 'pool' estimations are to be correlated with one another. A revolutionary advance in the study of the role of viral deoxyribonucleic acid in viral protein synthesis was reported by G. Brown, who appears to have succeeded in inducing *in vitro* the formation of small but significant amounts of phage tail antigens by addition of purified phage deoxyribonucleic acid to preparations of disrupted bacterial cells. Brown has already carried out extensive control experiments to establish the reality of the effects produced in this system, which, if confirmed and developed further, bids fair to become a biochemical philosopher's stone. In the discussion of the replication of deoxyribonucleic acid, F. W. Stahl summarized his and M. Meselson's experiment, in which the density of the deoxyribonucleic acid of bacterial cultures initially labelled with nitrogen-15 was examined after various periods of

growth in non-labelled medium (containing only nitrogen-14). Meselson and Stahl have shown by means of this procedure that the replication of bacterial deoxyribonucleic acid follows a semi-conservative course. That is to say, exactly as envisaged by J. D. Watson and F. H. C. Crick, the two complementary polynucleotide strands of the deoxyribonucleic acid macromolecule appear to separate from each other for the replication act and join up with single, *de novo* polynucleotide chains to form two replica molecules, each containing one old and one new strand. The capital importance of this convincing experimental confirmation of the Watson-Crick proposal is obvious. It is hoped that one may know soon whether these results can also be extended to the replication of the phage deoxyribonucleic acid.

The work of many phage geneticists in recent years has indicated that genetic recombination is a process which must be intimately associated with the replication of the hereditary material, namely, deoxyribonucleic acid. It is therefore of great interest that C. Bresch presented a new analysis of the population dynamics of genetic recombination in phage, elaborated independently by himself and by F. W. Stahl and C. Steinberg. This treatment represents a generalization of the Visconti-Delbrück theory, not only in that the nature of the elementary recombination act is not presupposed but also in that the pairwise mating-event of the earlier theory is replaced by a 'co-operation' in which the number of participating particles may be greater than two. A generalization of this sort had become necessary for two reasons: first, studies by Bresch and others have revealed a number of facts which do not agree with the Visconti-Delbrück assumption that genetic exchange in phage occurs by breakage and reunion of homologous parts of mated parental structures. Secondly, Bresch has shown by means of a triparental cross that more than two particles 'co-operate' in genetic exchange, so that mating is *not* pairwise. The implications of the demise of pairwise mating for the understanding of the molecular basis of genetic recombination are not yet clear.

In connexion with this same problem, R. Edgar and A. H. Doermann discussed new experiments pertaining to heterozygotes, those unstable phage genotypes which harbour alleles from parents of a mixed infection and which C. Levinthal first proposed as the transient zygotes formed at every elementary recombination event. Edgar concludes from crosses of several closely linked genetic loci that heterozygotes are indeed formed at the moment of genetic exchange. Furthermore, negative interference (that is to say, multiple exchange between three or more closely linked markers at a frequency greater than that expected from linkage data of two factor crosses) occurs at the time of the segregation of the heterozygote. In order to gain further insight into the genetic structure of the heterozygote, Doermann made crosses involving three closely linked markers and compared the frequency of progeny heterozygotic only for either one of the outside loci with the frequency of progeny heterozygotic only for the inside locus. Contrary to the 'overlap' model of heterozygote structure, now favoured by many phage geneticists, which predicts that phages heterozygotic for only the inside locus should be much less frequent than those heterozygotic for either one of the outside loci, Doermann found that all three types appear in roughly equal yield.

Although it is now evident that both negative interference and heterozygosis must hold one of the keys to the understanding of the nature of the elementary recombination act, it is difficult to assess how soon a full comprehension of these phenomena may be at hand.

S. Benzer reported his work on mutation in a single genetic region, or 'cistron', of the bacteriophage. By mapping hundreds of independent mutants produced either spontaneously or under the influence of certain mutagens, he has constructed a mutational spectrum and thus obtained an image of the relative mutability of individual loci within a single gene. The spectrum of spontaneous mutations shows a few 'hot' spots at which mutants recur with high probability and a number of other sites mutating with lower probability. The spectra of mutation induced by 5-bromouracil, 2-amino-purine, or proflavine, all of which raise the overall rate of mutation in the cistron by two orders of magnitude, are very distinctive, in that each of the three mutagens creates its own set of 'hot' spots and mutable sites, totally unlike the set of mutable loci identified either among spontaneous mutants or among mutants induced by the other two mutagens. Bromouracil, amino-purine and proflavine, therefore, are specific mutagens, in the sense that each raises the probability of mutation only at definite genetic loci, rather than causing a generalized mutability of the entire genome. Since concrete notions concerning the mode of action of each of these mutagens can already be formulated in terms of the Watson-Crick structure of deoxyribonucleic acid, mutational spectra promise to bridge the gap between chemistry and genetics.

The past decade saw an ever-growing confluence of phage and bacterial genetics, particularly as non-infective prophage turned out to be an integral part of the bacterial genome in lysogeny, and infective phage a carrier of bacterial heredity in transduction. Recent studies on bacterial recombination, lysogeny and bacteriocines, presented at the Colloquium by J. Lederberg, by R. Frédéricq and by F. Jacob, have revealed, however, that the bacterial fertility factor *F'*, the prophage and the colicinogenic factor have certain features in common which set them apart from other, or 'regular' bacterial genes. On the basis of these special properties, F. Jacob and E.-L. Wollman have created a new concept, the 'episome', defined as an exogenous hereditary determinant integrated into the bacterial genome by addition to the chromosome instead of replacement of allelic bacterial loci. Besides its integrated chromosomal state, the episome can also exist in an autonomous state, in which it is replicated independently of the rest of the bacterial genome. The episome promises to bring new order into the dialectics of virus-host genetics.

The second International Phage Colloquium can be considered to have been a great success; not only the participants but also phage workers in general must be grateful to its organizers, A. Lwoff and F. Jacob, and to the Rockefeller Foundation which provided financial support. The week of intimate and informal living within the historic Cistercian monastery founded by St. Louis in the thirteenth century once more promoted understanding, collaboration and personal friendship of a truly international nature. There seems little doubt that this Colloquium, like its predecessor, will have a profound effect on the development of bacteriophage research in coming years.

GUNTHER S. STENT