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Chapter 3

Genetics and Microbiology[1]

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"To the biologist of the 19th century, bacteria appeared as the most primitive expression of cellular organization, the very limit of life. In reality it appears that it is only their small size and the absence of recognized sexual reproduction which has given the illusion that bacteria are simple cells." Thus Dubos (11) introduced an outstanding synthesis of the structural and biochemical complexity of the microbe as a living system. But in another realm, he (12) could not repress a nostalgia for primitive simplicity: "Bacterial variation passes from the collector's box of the naturalist to the sophisticated atmosphere of the biochemical laboratory. One may wonder whether the geneticist will not arrive too late to introduce his jargon into bacteriology."

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Alas, it could not be helped; the same symposium (Cold Spring Harbor, 1946) was already teeming with cytogenes, mutagenesis, allelomorphs, recombination, and heterozygotes, and microbiology had already succumbed.

To an impatient bystander this fusion—or confusion—of disciplines might seem overdue. Mendel was, after all, a contemporary of Pasteur, and could we not imagine their communication and understanding that the abbot's "Anlagen" were the stuff of unspontaneous generation? But Pasteur was a prophet honored in his own time, while Mendel was first ignored and then forgotten, and the burgeoning science of microbiology grew up without benefit of eugenic supervision.

As a science, genetics dates from the exhumation of Mendelian analysis at the turn of the century; as a term, it was first coined in 1906. Genetics might have been infused with microbes from the start, with Blakeslee's discovery of the segregation of fungal sex factors in 1904, had the zygospores of the mucors germinated more readily, but he turned to more amenable plants for his genetic work, and he works with them still.

Heredity has always been an object of avid curiosity and inquiry. Darwin, for example, was obliged to consider some mechanism of inheritance to underlie his evolutionary theory and, with some misgivings, adopted a Lamarckian concept whereby the progeny resemble their parents whether for innate germinal, or incidental somatic peculiarities. This fallacy was eroded by Weissmann and (for higher organisms) laid to rest in the 1900's with the Johannsen and de Vries delineation of pure lines and their mutations. Prior to Mendelism, strong circumstantial evidence already supported widespread belief that the chromosomes were the primary vehicle of heredity.

Meanwhile, the bacteria were and remain a convenient repository for hypothetical evolutionary starting points and speculated genetic mechanisms that might be refuted

by Mendelian and populational analysis elsewhere. To be sure, such pioneers as Massini, Beijerinck, and Barber did distinguish fluctuating or impressed variations, which are reversible physiological responses to the environment, from fixed mutations representing innate genetic alterations. Bacterial mutations are reversible, however, in the same sporadic fashion as the primary events; reversion is no less characteristic of other organisms, but this was overlooked in support of cyclogenic or more obscure special theories of bacterial dissociation (18). The confusion was compounded by the tremendous size of bacterial populations and the aggressiveness with which bacterial mutations and reverse mutations often present themselves (6, 33).

These features were put to good use in a renewal of exact study by a biometric approach in the 1940's, culminating in the analysis of clonal variance of mutations to phage resistance by Luria and Delbrück (34). The outstanding qualitative result of these experiments was statistical proof of the uncontrollability, if not the absence, of purposive regulation of adaptive mutations, such as resistance to phage or to streptomycin. The statistical procedures have since been expanded for the calculation of mutation rates and for more detailed analysis of spontaneous mutation (2, 38). But population dynamics is so complex (7) that foreseeable progress here may consist of balancing the complications omitted from the approximate calculations against the imprecisions of measurement. The clonal variance that is the keystone of the qualitative argument for preadaptive mutation also requires tedious repetition to measure mutation rates with a scarcely acceptable precision of $\pm$ 50 per cent (34, 38, 42). Ordinary cultural methods also entail an ever-changing chemical environment (7) in addition to an inexhaustible reservoir of systematic as well as sampling errors (26).

Novick and Szilard (39) have resolved these obstacles by means of a simply engineered continuous-flow device,

the chemostat, in which cultures can be maintained in a self-regulated, steady-state environment long enough to submerge short-term random fluctuations. Accordingly, spontaneous mutations can be measured with a precision of 5 to 10 per cent and an assurance that nonlinear perturbations of the steady state are either self-cancelling or so exaggerated as to be self-evident. Sophisticated studies of bacterial mutation can no longer afford to ignore the experimental control uniquely offered by this approach.

"Experimental control of spontaneous mutation" is an intentional paradox. "Spontaneous," despite purposeful misreading by dialectic materialists, means neither the immateriality of the gene nor the notion that genetic material lacks a physical nexus with the environment (19). It does mean that the subtle chemistry of the gene has evolved through conservative mechanisms so refined that we cannot discern their connection with the end products of organic structure and function; at least we have not yet learned how to distinguish one gene from another by reactants that allow purposeful, specific changes. Some day the secret of specific mutagenesis will be revealed, but such faltering claims, for example, as that antibodies might alter specific genes have not held up (42). To my mind, the search for this philosopher's stone by bludgeoning the bacterial gene with drugs or enzyme inhibitors will some day seem as credulous as the snipping of mouse tails does now, and it has already had equally negative, if sometimes glittering, results. [I cannot overlook the remarkable effects of one inhibitor, acriflavine, on respiratory factors in yeast (13), but this seemingly embarrassing exception has illuminated the path from infection to heredity (9, 28)—the effect is akin to the "chemotherapeutic cure" by streptomycin of green plants with regard to their chloroplasts (41, 48).]

Experiments with the chemostat have demonstrated significant environmental control over rates of sporadic muta-

tion of various bacterial genes. Not only a rise in temperature, but also such metabolic variables as tryptophane deficiency or adenine excess accentuate the mutation rate (40). Perhaps the most provocative finding was that ribonucleosides would not merely reverse the effect of excess adenine but would reduce the "spontaneous" rate by half (39). Biophysicists once speculated, and then rejected, cosmic radiation; we now observe that the causation of spontaneous mutation is a problem incidental to intermediary metabolism.

This conclusion converges with the explicit study, largely with microorganisms, of mutations induced by ionizing radiations and chemical reagents. "Induced" may be as misleading as "spontaneous"; it implies contrived increase of the over-all mutation rate, in a sense, an augmentation of "spontaneous" change. Between 1928 and 1940, the only accepted mutagen was radiant energy, many chemicals having been inconclusively or at least unconvincingly tested (3, 36). But starting with the war gases, and now including such domestic articles as hydrogen peroxide, formaldehyde, soporific drugs, and caffeine, so extensive a catalog has been shown potent (3, 10) that one wonders what, besides rigidity of concept, can have hindered the germination of chemical mutagenesis for so long. Now, from a free interchange of results concerning microorganisms and macroorganisms, ionizing radiations also are inferred to work through chemical intermediaries: free radicals from oxygen and water (45). And, first with *Streptomyces* (22), then with other organisms, the dysgenic effects of ultraviolet have been found reversible by visible light, suggesting an obscure photochemical intermediation.

Mutagens have, of course, helped to furnish variants, the raw material for other experiments. But, particularly in industrial applications, there has been exaggerated emphasis on the technology of provoking genetic variation (which

is relentless anyhow) at the expense of thoughtful search for procedures to detect and electively enrich the variants that serve some specific purpose, an approach we have inherited in large part from the Delft school of microbiology. Some helpful methods have been developed (32) for the isolation of the biochemical variants that Davis has so skillfully exploited.

Genetic Recombination

All this bears more heavily on the general utility of microorganisms in genetic research than on the genetics of particular microbes. Once given that microbes are differentiated into more than the rhetorical "bag of enzymes," as already proved by mutation research, we must look more deeply into the organization of different microbes, and for this, *genetic recombination* is the most versatile tool now at hand. After all, most experimentation consists of putting together two reactants and waiting to see what happens. Synthetic chemical reagents are too crudely designed for us to tell very much about their ultimate effect on genetic configuration, and we therefore seek the means of combining the biological units themselves. Later, if we can keep busy, patient, careful, and lucky, we may hope to build genetic theories on cytological facts, but the present foundation of unarguable correlations is still unsteady, perhaps owing to technical difficulties as much as to the unavoidable subjectivity of cytological conviction. If they are cells, bacteria do have organized nuclei and some approximation to mitosis, but we lack the very criteria of proof on which we can readily judge current controversies on particular manifestations of these forms.

Genetic recombination includes any process of the coalescence within one cell or organism of genetic factors from two or more parents. Its best known manifestation

is sexual fertilization: a union of an entire gamete nucleus from each parent to form the hybrid, zygote nucleus. There are, of course, innumerable secondary physical and psychic paraphernalia to support the act of fusion, but this and not the accessories is the essence of sex for genetic purposes. Sex occurs universally among higher plants and animals and is nearly as prevalent among protozoa and fungi, but, with sporadic exceptions, has been reported absent from the bacteria.

Among lower plants, we also discern another mode of recombination—heterokaryosis—in which the interaction of intact nuclei falls short of fusion. Instead, diverse nuclei multiply *sui generis* in a common cytoplasmic pool, where their functional contributions are so intermingled as to simulate hybridity. By sporulation, accident, or surgery, however, the nuclei of the heterokaryon may be segregated to reveal their lasting integrity. In the higher fungi, a binary heterokaryon is a regular feature of the life cycle, is maintained by conjugate mitosis, and may be ultimately terminated by sexual fusion, whereas in the ascomycetes and the phycomycetes, the nuclei of heterokaryons multiply independently. A similar type of heterokaryosis almost certainly occurs in actinomycetes (27); among nonfilamentous bacteria it could only be transitory, but cannot be ignored in the momentary control of phenotypes in mutation and recombination procedures.

By 1945, morphological approaches to the question of bacterial sexuality had raised so many vexatious controversies that, to quote Dubos' book (11) again: "If bacteria do really reproduce by sexual methods, it should be possible to cross closely related species and strains . . . most workers who have attempted to cross related strains have reported only failure."

Hindsight suggests that the chief omission of previous experiments (14, 43) was a set of clear-cut, unit markers in a selective system that would allow the detection of in-

frequent recombinants. But as early as 1908, Browning (8) did, in fact, make an impeccably designed test of sexuality in trypanosomes, using drug resistance for selective markers. In 1946, this forgotten experiment was repeated with some strains of *Escherichia coli,* and has since provided grist for the mills of several laboratories (30, 46). The main conclusions of the recombination analysis point to the participation of intact cells in a sexual interaction, and it is called sexual because the putative gametes encompass the whole genetic content of each parent (37). Selective methods were originally necessary because of the infrequency of recombination, which also precluded a parallel morphological decision.

More recently, especially favorable strains with higher fertility have facilitated microscopic studies, and in appropriate mixtures of cells conjoined pairs have been seen and isolated with the micromanipulator (27). If left undisturbed, the swimming pairs will disjoin in an hour or two. Exconjugants from about half the pairs engender detected recombinants (and other sexual progeny are doubtless undetected with the particular markers used). The recombinants issue from only one, the maternal parent, so to speak, to imply that conjugation transfers a nucleus from one cell to the other, followed by fertilization and reduction, rather like either half of a paramecium mating. No distinctive zygote structures, aside from the pairs, have been noted. Except that it submits to considerable stretching and torsion, nothing has been seen directly of the conjugal apparatus, probably because of optical limitations. A good deal remains to be learned, but I want to emphasize that this experiment is validated primarily by the genetic, not the morphological, observations.

Although about 5 per cent of *E. coli* strains are known to be fertile, sexual recombination has not been verified, or at least studied genetically in any recorded detail, in other bacteria, though a number of leads are being in-

vestigated in several places. Genetic exploration of various morphological representations of bacterial sex is overdue.

Genetic Transduction

We now depart from mechanisms that should be familiar to every student of general biology, and must deal with a unit not previously recognized in genetics: the hereditary fragment that defines *genetic transduction.* In 1928, Griffith transformed the capsular specificity of rough pneumococci by heat-killed vaccines of smooth types (16). One is again forced to acknowledge the fortuitous success enjoyed by some irrationally designed but well-executed experiments. Although not cited by Griffith, the literature of the preceding decade (26) carried many accounts of paragglutination, whereby a superficial attachment of heterologous antigens to bacterial cells was misconstrued as hereditary alteration. Griffith, however, soon realized (as some of his successors have not) that the transformation could not concern merely the capsular polysaccharide, but must involve the machinery for its formation, as we would now say, a "genetic" or metapoietic factor. Griffith also conceded the theoretical qualification that his vaccines contained residual bacteria not revealed by conventional sterility controls, but resuscitated in the experimental mixtures. This caution has often been overlooked and can be disposed of only with the help of strains differentiated by several markers (23, 24), as was eventually done with the pneumococcus also (4, 20).

Unfortunately, the occasional notice taken of Griffith's work by genetically minded workers was often confused by the prescription of "directed mutation" and perhaps by the indiscriminate use of "transformation" for any species of change; it was not for another twenty years that this transformation was again generally accepted (36) as

an example of fragmentary genetic transfer, that is, *transduction.* Meanwhile, the chemical analyses by Avery and his colleagues described the reagent in the vaccines as principally, if not exclusively, desoxyribonucleic acid, DNA (4). The genetic aspects of the transmitted fragment have not been fully clarified, but in each of the several examples of transduction, single genetic factors are the rule, punctuated by occasional "linked transduction" of two factors (20, 31, 44). As the number of factors examined increases, so does the incidence of recognized linkages. This suggests that the unit is a chromosome fragment, rather than an absolutely delimited macromolecule, the idealized "single gene."

Transduction in at least two other bacteria, *Hemophilus* and *Neisseria,* was discovered by conscious emulation of the Griffith and Avery procedures, and with some advantageous peculiarities, its general aspects are the same as in the pneumococcus (31). In *Salmonella,* on the other hand, transduction was accidentally discovered in the course of a fruitless search for sexuality as it occurs in *E. coli.* In fact, too rigid insistence on the use of double mutants to control the selection of recombinants nearly obscured the initial discovery (49), but this emphasizes the difference in mechanism. Transduction in *Salmonella* differs from that in the pneumococcus primarily in the function of temperate bacteriophage as the passive vector of the hereditary fragments.

To recapitulate, genetic transduction as much as sexual fertilization is an agency of recombination but differs in two principal features: morphologically, one reactant is a subcellular fragment, and genetically, perhaps as a corollary, a small fragment rather than the entire genotype is all that is transmitted. In several bacteria, the fragment may be transmissible after chemical extraction in essentially native form, possibly pure DNA, but in *Salmonella* a symbiotic phage effects the initial fragmentation of the

bacterial nucleus, the intercellular transport of the fragment, and its injection into the new host bacterium.

Insofar as we elect to regard hereditary viruses as part of the genotype, symbiotic infection is also a species of recombination. Lysogenization in particular can be analogized to transduction or even fertilization (15), especially since, at least in *E. coli* K-12, the prophage is incorporated as if it were a typical genetic factor (25). In addition, traits usually attributed to the "bacterium itself," whatever this means, may be dramatically converted by lysogenization *per se,* as in the toxigenic variation of diphtheria bacilli (5, 17) and the change in lysotype (1) or somatic antigen from group E-1 to E-2 (21, 47) in *Salmonella.*

It is less urgent to distinguish whether lysogeny is a transduction, which is mostly semantic manipulation, than to describe the role of the phage particle. This may be considered as a miniature bacterium, with a skin and a "nucleus." The phage nucleus itself is the agent of genetic conversion in lysogeny; it behaves rather as if it were a special segment of a bacterial chromosome, but as with any virus we cannot say whether this evolved by gradual parasitic degeneration or by abrupt mutation.

In addition to the phage nucleus, the skin may enclose other fragments, the residue of the lysed bacterium. The relationship of these fragments to the prophage in *Salmonella* is obscure. The simplest picture is that they are adventitiously included, together with the phage nucleus, during the maturation of the phage particle. However, it has not yet been possible to study the localization of prophage in *Salmonella* by the methods employed for *E. coli* K-12, and it cannot, therefore, be excluded that the fragments transduced by any given phage particle are related to a less rigidly predetermined reproductive site of the nucleus of that particle. In any event, every genetic factor so far tested in *Salmonella* is subject to transduction, although the quantitative efficiency may vary by as much as

fiftyfold from one factor to another. Another phage-mediated transduction has been found in *E. coli,* but this is rigidly limited to a cluster of factors (for galactose fermentation) closely linked to the prophage site (25, 35), and a special relationship of the transduced fragment to the developing phage is therefore certain.

The distinctive features of phage-mediated transduction in this context are: (a) the transductive competence of any crop of phage is determined entirely by the genotype of the host cells from which the crop is obtained, and (b) lysogeny is separable from the transformation, that is, transduction may be consummated without the necessary establishment and maintenance of the lysogenic state, and recipient bacteria may be lysogenized without usually manifesting other genetic changes. In the lysogenic conversions, the competence of the phage is essentially independent of the host, and lysogeny is both necessary and sufficient for the concomitant changes. It is conceivable that these conversions are a relic of "bacterial" genes not yet redifferentiated in the phylogeny of the phage, but the chief virtue of such ethereal speculations is to emphasize the ambiguity of our concepts of organismic individuality (8).

Potentialities of Recombination Methods

Why emphasize the prospects of recombination over other means of genetic analysis? First of all, it should lead to the substantiation of life cycles (compare alteration of generations in plants and animals), but we must confess that the L-forms have eluded genetic analysis. Then, recombination is indispensable for understanding other modes of genetic variation. For example, it furnishes proof that the effects of acriflavine in yeast, already noted, are cytoplasmic depletions rather than directed gene mutations, while in lysogenic *E. coli* it has fixed the genic locali-

zation of the prophage, which had been thought a likely inhabitant of the cytoplasm.

Recombination also gives logistic support to other experimentation, for example, in biochemistry or immunology, by allowing the rational construction of prespecified combinations of genetic factors. The potential of recombination methods in applied microbiology should be so obvious as to obviate comment but has generally been overlooked in favor of more routine screening methods.

Finally, but not exhaustively, the very occurrence of recombination illuminates both taxonomy and evolution, for example, in rationalizing the otherwise unintelligible list of serological types of *Salmonella* (29). These findings, in turn, may reopen the question of how much reliance should be placed on serological typing of *Salmonella* as a clinical rather than an epidemiological tool. Although distinctions between serotypes are entrenched in the public health laws of many states, supposedly innocuous serotypes such as *S. typhimurium* are too often ignorant of the legal definitions of paratyphoid fever.

To offer seriously any prescription for the future of science would require a calculated blend of presumption and inattention to history. If such predictions were accurate, we would be disappointed, for they are the measure of the bounds of current thought that we hope to overreach. The perspectives of current microbiological science might be mistaken for prophecy; but the wisest prophet would look beyond the visible horizon for the questions we are not yet ready to ask.

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