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Lilly Award Lecture

This is, I believe, the first time that the Society has assigned a geneticist to the pleasant burden of this lecture. ~~This may be read as evidence that bacterial genetics has only recently attained its majority.~~ It may be pertinent to summarize some of the historical traces of bacterial genetics: ~~Although we are too close to its adolescence to~~ without presuming to any premature, we can any perspective ~~wisdom, it is possible to~~ trace some of the ~~most important invigorating elements of its hybrid~~ ancestry. ~~They number:~~ First, the extension of elective enrichment as a fundamental tool for the isolation and enumeration of specific microbial types within initially pure cultures, as well as from the mixed flora of natural habitats; Second, the demographic or populational treatment of bacterial cultures and colonies as aggregates of individual cells, whose homogeneity must be explicitly assessed, usually by just such selective methods; and, Third, the realization that the very individuality of a bacterial type poses a problem in heredity, which must be met and answered in terms of some genetic theory. ~~The correspondence of a competent theory for bacteria with the~~ structure that has been developed ~~for higher organisms has~~ could be and ~~been verified by experiments~~ on various bacterial species. ~~But, historically, and analogically,~~ we may recall that the studies with Neurospora, which exposed metabolic ~~peculiarities as the consequences of gene mutations, were a potent stimulus~~

~~to related inquiries with various bacteria.~~

These three methodological principles have been fruitfully applied in bacterial genetics as in the analysis of different modes of adaptation and of agents which will induce genetic mutations, but I must confine my remarks today to their elucidation of the exchange of hereditary determinants among bacterial types.

Even today, there is no compelling morphological evidence of sexual fusion in bacteria--a remark that applies a forteriori to the pictures I will show myself, later. Many other published claims have been supported by highly suggestive, but not irrefutable photographs. My own account starts about seven years ago, By which time Most thoughtful students had concluded that a purely morphological approach was unlikely to be decisive, and the desultory attempts to detect crossing by genetic techniques had given results either negative or incredible. At the least, the burdens of proof devolved upon any affirmative claims of bacterial sexuality. Some favorable circumstantial evidence did, in fact, encourage the first experiments. In one of the most thoroughly categorized groups of bacteria, the Salmonellas, the patterns of the various somatic and flagellar

antigens represented in the Kauffmann-White scheme are scarcely intelligible except in terms of recurrent recombination. As we shall see, this conjecture has been confirmed, though not quite as expected.

Escherichia coli was a preferred species for the first investigations. In 1944, E. L. Tatum (and, independently, R. R. Roepke and his associates) had isolated nutritionally exacting, what we now call "auxotrophic," mutants from strain K-12 of E. coli. In 1946, I went to Professor Tatum's laboratory at Yale University to join in experiments we had previously discussed to test the possibility of genetic exchange with the help of such mutants. We planned to exploit the selective property of a synthetic, minimal medium to suppress auxotrophic mutants, and thus to select for prototrophic bacteria--those with wild type nutrition, and lacking differential growth-requirements. Genetic exchange could be efficiently detected by culturing different auxotroph mutants together in various media, and then plating them into minimal agar. The parental auxotrophs would be suppressed, but any crossing should also engender, among others, prototroph recombinants which would be readily detected and recovered. The selective efficiency would so far exceed crossing experiments previously reported (for example by Gowen and Lincoln), that these negative results would not necessarily

be discouraging.

The first experiments gave an inescapable result: mixtures of various auxotrophs would persistently engender prototrophs in the ratio of about a million-to-one. Clearly, the key to this affirmation is the selective method; otherwise, it would have taken far more time than you would care to listen about to conduct the experiment to a single definite results, or more likely it would have been given up as a hopeless task, like its predecessors.

It was necessary and possible, in various ways, to confirm that the prototrophs were pure cultures, and that they could not be explained as an artefact of spontaneous variation of either parent by itself. But the generation of prototrophs was only the first stage of the analysis, showing that some form of genetic interaction between different bacteria was possible. The behavior of other specific traits or genetic markers and the physical and cultural conditions of its occurrence must next be recounted.

E. coli is one of the most ubiquitous of bacteria, and the characteristics of any typical strain will be familiar to each of you. Except that during the thirty years since it was first isolated it may have lost

characteristic O and K antigens, strain K-12 conforms to type. In particular, it exhibits no special growth requirements, it ferments a variety of sugars (glucose, lactose, maltose, xylose, mannitol, and so forth, but not sucrose or cellobiose), it is susceptible to many "coli-phages" and colicins and other antibiotics, including streptomycin. These characters are enumerated only to indicate the traits which, in suit of genetic variation, have furnished the genetic markers for further studies. ~~During the past several years special methods have been developed to facilitate the isolation of biochemical variants; mutants leading to resistance to phage or streptomycin are, of course, most readily isolated by direct selection of large bacterial populations.~~

If they are generated by a sexual process, the prototrophs should also exhibit recombinations for any additional unselected markers which might differentiate the two parents. For example, if one auxotroph parent were lactose-positive and streptomycin-sensitive, while the other auxotroph were lactose-negative, streptomycin-resistant, the prototrophs should fall into four classes in respect to these two unselected markers: the parental combinations--positive-sensitive and negative-resistant--and two new combinations, positive-resistant and negative-sensitive. With three markers, there would be

eight potential classes, and so forth. This prediction has been borne out in great detail, some crosses having been carried out with as many as six and seven differential markers. Moreover, the role of a marker as selected or unselected is not absolute, but depends on the technical details. In a medium supplemented with the appropriate growth factors, selection on the nutritional markers may be relaxed, while bacteriophages and antibiotics may be substituted in an obvious way as the specific selective agents. Thus it has been possible to recover some of the recombinants that would otherwise be missed by prototroph selection, such as dual auxotrophs. The regularity with which an unlimited array of recombinants can be generated, regardless of the particular mode of selection, refutes their interpretation as any artefact of spontaneous variation. Theory and experience concur again that recombination does not generate any new variation beyond the reshuffling of markers already embodied in the parents. In all genetic work it is, of course, essential to scrutinize any marker for its inherent stability and the regularity with which it can be classified. Special attention must be given this point in recombination studies, but if this is satisfied, the unrestrained reassortment of unselected markers is the surest testimony of a recombination process.

If, among the prototrophs from a given cross, the various classes of combinations of markers are enumerated, it is found that they appear in characteristic proportions: although, as a rule, every possible class will be represented, some combinations will be much more frequent than others. As a general rule, parental combinations will be more frequent than recombinations for any small group of markers. Continued study reveals many very marked correlations of pairs of factors. For example, with lactose-fermentation and T6-resistance, the two recombination or "crossover" classes together made up respectively only 6% and 2% of the total, while the two parental combinations were 75% and 17%. Similarly, for many other pairs of factors ~~for maltose-fermentation and streptomycin-resistance; xylose and mannitol fermentation; threonine and leucine-requirement; and thiamine- and methionine-requirement are similar pairs of closely linked markers.~~ By an extension of this type of analysis, it has been shown that at least six or seven markers (four already mentioned, and one for resistance to phage T1) can be ordered on a linear linkage map. On such a map, the probability of recombination between two markers is proportional to the indicated distance between them; it is linear insofar as these probabilities are additive. The triumph of genetics has been

the rigorous correlation of the linear linkage map with the linear chromosome. A proof of equal rigor has yet to be accomplished in bacterial cytogenetics. The mapping of these factors, beyond the pairwise relationships already mentioned, may be complicated by various anomalies of "chromosome" behavior which may or may not be of primary importance. Temporarily suppressing any such anomalies, we may summarize the customary life cycle of strain K-12 (as witnessed by genetic evidence only) as follows: the vegetative cell is haploid, although other genetic and cytological work supports a two or four nucleated condition as usual. Among a million cells, under ordinary cultural conditions, a single pair may mate by a process still unobserved, though a full cell fusion is perhaps less likely than a ~~more limited~~ temporary conjugation. The diploid stage is evanescent, and persists only long enough to allow reassortment and the segregation of haploid recombinants to complete the cycle. This will be recognized as following the same sequence as many other fungi,--Neurospora or Zygosaccharomyces,--and dissimilar to the yeast Saccharomyces which has a prolonged diploid phase.

The experiments so far tell nothing of the chemistry or morphology of the mating process. Two alternatives merit the closest consideration:

a bona-fide union of two cells, or something akin to the pneumococcus transformation. This second alternative would mean that one of the parental gametes would be replaced by a sub-cellular fragment. However, extensive studies, in several laboratories, have uniformly failed to substantiate the second alternative; the only conditions which permit recombination are those in which direct access is permitted between the parental cells. Hayes has found that cells that have been, so to speak, "killed" with streptomycin may function (with reduced efficiency) in recombination. A separation of the capacity for colony formation from other signs of vital function has, however, many precedents in disinfection studies. Morphological changes in the streptomycin-treated material to substantiate a gametic role for any element other than the entire cell have not been presented. Other antibacterial agents do not markedly discriminate between sexual and vegetative functions, but It is very difficult to evaluate many of the experiments that have been published on these effects. As predicted from the mating theory, recombination has been shown, by T. C. Nelson, to fit the kinetics of a bimolecular reaction, and rates of recombination can be compared quantitatively only when the rate constants can be inferred. But no amount of negative evidence can add up to an affirmative picture of the

details of the mating mechanism. Until a morphological demonstration is completed the hypothesis of a conjugal union to explain genetic recombination has this weight only: that it is consistent with every datum so far adduced.

Until recently, morphological study had no encouragement whatever. Nelson's kinetic constants could be read as counting about 5000 random collisions for every mating, a rate simply too low for any but speculative cytology. Subsequently, L.L. Cavalli discovered a much more fertile strain, with which the ratio of matings to random collisons approaches one, so that constructive cytology is now possible, though still difficult, and the microscopic approach has been resumed. In some very early attempts, we have had some encouragement from seeing pictures like this.

LANTERN SLIDE 1

What such figures may have to do with the mating process is purely conjectural. It is tolerably certain from phase-contrast microscope observations that cells may be attached in pairs while living, but their further history has not been followed up. We have seen nothing else, so far, with any suggestive quality. The outcome of this study will, we feel, be of public interest as exposing either an artefact of which we must beware in all such

studies, or as some aspect of the sexual mechanism. I want to emphasize that, by itself, any picture such as this stands as very meager evidence indeed.

I have already indicated that the postulated diploid, zygote phase is short-lived, and is not propagated as such at all in the usual sequence of the life cycle. This diploid phase, is in fact, a figment of ~~reasons~~ inductive ~~in this~~ inference from the facts of recombination. Fortunately, exceptional deviations from the standard life cycle have substantiated this reasoning, for un-separated (or non-disjunctional) diploids have been found among the progeny of a certain mutant stock, "Het." When a Het Lac+ is crossed with a lactose-negative parent, many of the progeny are typical prototrophs, stable both for their nutritional and their fermenting qualities, though, of course some will be "Lac+" and others "Lac-." A few percent, however, prove to be persistently segregating for all of these qualities (and indeed for almost any marker that may distinguish the parents). This is ~~shown in part by~~ their appearance on an indicator medium:

LANTERN SLIDE 2 = Fig. 2, p. 420 C.S.H. 1951

which is typical of the colonies stemming from a single cell. To abbreviate a long story, the heterozygous diploid cell is segregating for several markers.

The stable, haploid segregants usually display the combination of markers of one or other parent; less often, new combinations are seen. ~~However,~~ The markers always segregate at the same time. The facts of recombination agree ~~nicely~~ with the hypothesis that the recombinants stem from intermediate diploids like these. What is exceptional here is the tendency of the diploid cell to propagate as such, in about 19 out of 20 fissions, in M. R. Zelle's single cell pedigrees. It is thus possible to compare the nuclear cytology of haploid and diploid cells:

LANTERN SLIDES 3 - 4 = 56.6 p.427 C.S.H. 1951

About all that can be surely claimed in the present state of bacterial cytology is that the diploid cells have distinctly more complex nuclei. I would not venture, as yet, to quote chromosome counts on this material. ~~To mitigate possible misunderstanding,~~ It should be emphasized that the diploid hybrids are not merely unstable for a single marker, but show a bloc-wise separation of all of the numerous markers differentiating the two parents. Two markers have to be excepted from this rule: maltose-fermentation, and streptomycin-resistance. These have been invariably hemi-zygous, i.e., only once represented, in the diploid progeny of Het

crosses, although sometimes one, sometimes the other parent's markers are preserved, in any given diploid. This raises the question whether the gamete was already defective, or whether there has been a later elimination of a chromosome segment carrying these linked markers. The second interpretation that the gametes are intact and the aberration secondary is favored by ~~the occurrence of some~~ diploids in which the Mal marker comes from one parent, the streptomycin marker from the other.

For some time, K-12 was the only E. coli strain in which recombination could be demonstrated, others having been tested with negative results, but in a later survey about one wild-type strain for every 25 tested was found to be fertile with strain K-12. The fertile strains encompass a wide variety of serological and cultural subtypes, but all of them are included in E. coli as presently understood. The taxonomic delineation of this species is therefore supported by concrete genetic evidence. Whether the other 24 out of 25 strains form additional intra-compatible groups is not known, but entirely possible.

One purpose of this survey was to see whether compatibility preferences could be found by ranging over a large group of strains. But meanwhile, it was found that the social structure of K-12 was not so simple either. All

of the crossing stocks originally had been developed from the same clone of K-12, and since they were all crossable with each other it was concluded that the strain was not (to speak loosely) sexually differentiated, i.e., it was homothallic. Later, Cavalli (in Milan) and Mrs. Lederberg (in Madison) discovered certain K-12 strains to be both self- and mutually incompatible. We called the wild type, compatible strains, F+; the incompatible, F-, and to recapitulate, F- x F- is sterile, while F+ x F+ and F+ x F- are both fertile. Therefore, it was not until two F- testers showed up that the incompatibility system could be uncovered. About the same time, Hayes had found his differential effect of streptomycin, and a comparison of all our results indicated also that F- cells were completely inactivated by streptomycin, while F+ cells retain some sexual function. The speculation that the F- cell donates the larger part of the cytoplasm to the zygote, i.e., that it may be a sort of oo-gamete, is still tenable. Other studies have also shown that the polarity of a cross with respect to F status also determines the trend of elimination of the deficient maltose- and streptomycin-markers from the diploid zygote.

If compatibility were inherited like other markers, it would have been detected long since. But, remarkably, it is contagious, for when properly

marked F- and F+ cells are simply grown together, after a few hours most of the F- are converted to F+. This conversion is rather mysterious. Although it occurs about as frequently as the calculated collisons of the two kinds of cells, no infective agent has been separated from either the F+ or F- sources. There is a rough (but not a detailed) agreement of the circumstances under which this conversion occurs, and the circumstances of genetic recombination, and indeed both may require a contact of cell surfaces. The supposition, advanced elsewhere, that the so-called F+ agent rather than the cell itself is the vehicle of genetic transfer, ~~cannot be studied~~ is impregnable until the two have been separated physically. Other differences can be accomodated by postulating a variable competence of the F+ agent or its infectivity and maintenance.

Among the new crossable strains about half are F- and can be converted to F+ by growth with K-12; others are F+ and will convert K-12 F- stocks to an F+ state of variable permanence. But we are far from knowing the whole picture; some strains show no signs of compatibility differentiation, or of the F+ agent, although ~~they~~ one can be "infected" with it. The only sign of this cryptic infection is that the infected strain can reconvert a K-12 tester. But the evidence for an F+ virus is purely epi-bacteriological,

and a term such as infection may be an unwarranted extrapolation, however convenient it is as a laboratory shorthand.

Despite the gaps in our knowledge, the foundations of recombination are already secure enough to allow applications to many problems of general interest. For example, numerous genetic factors are concerned with the economy of single bacterial enzymes, and vice versa, which show in turn that the one-gene : one-enzyme hypothesis is a useful, but fictitious approximation. In a preliminary study of antigenic factors in Escherichia coli, Dr. P. D. Skaar in our laboratory has shown that H, O, and K antigens may be recombined in the same way as other genetic markers differentiating different strains. And in the field of drug resistance, Newcombe and Cavalli have shown, on the one hand respectively, that the response to streptomycin is achieved, ~~in the main,~~ by a single genetic mutation which confers full resistance, while resistance to chloramphenicol is governed by the interaction of great many separate mutations with cumulative effects. These findings were powerful confirmation of previous anticipations. The results of a recent investigation by Mrs. Lederberg were less predictable. We had thought that symbiotically carried bacteriophage would behave like a cytoplasmic genetic factor, that is,

like one of the plasmagenes which are the topic of a good deal of geneticists' discussions these days. However, the results of crosses of lysogenic and sensitive sub-strains of K-12 showed that the trait of lysogenicity was inherited like any other marker, and that it was in fact linked to markers for galactose fermentation. The clinching evidence here was the isolation of diploids heterozygous for these markers, so that a hybrid which was lysogenic and galactose positive would engender segregants some of which are of the other parental type, sensitive to the potentially symbiotic virus and galactose-negative, together with a small proportion of the other two combinations. F. M. Burnet had long since foreseen that the lysogenic complex embodied the integration of the latent phage in the hereditary make-up of the bacterium, but it is difficult to see how this concept could have been substantiated more firmly than by recombination analysis. But I would still conclude that the largest contribution of this approach is the impetus it gives to the unification of bacteriology within a more coherent comparative biology.

This is not to say that there is nothing unique in bacterial genetics: since 1928 the pneumococcus transformation has stood to refute any such complacency.

As soon as the outlines of the K-12 story became visible, it was important to learn whether sexual recombination occurred more generally among bacteria. The Salmonella group was the next choice of material. My early experiments with S. typhimurium gave the tantalizing result that various auxotroph mixtures appeared to give prototroph recombinants, but that attempts to secure evidence of recombination of unselected markers all failed. Therefore, I drew what later turned out to be the skeptical but incorrect conclusion, that these apparent prototrophs were artefacts, and ~~that~~ more different strains had to be studied. When N. Zinder joined the program, this was his experience also for over two years, and crosses of over a hundred pairs of parents. It was only when we incorrectly interpreted a two-step mutant as a two-factor mutant that we drew the correct conclusion, that a recombination mechanism was in fact operating. This system proved to be very different from the sexual recombination that we had been looking for along the lines of K-12 work. Instead, genetic transfer here is mediated by a filtrable agent, namely certain potentially lysogenic bacteriophages. When this phage is grown on one Salmonella strain, some of its markers can be transferred by the phage to a second bacterium,

and there replace the previous markers of the recipient strain. In general, only a single marker is carried by a given phage particle, and the over-all efficiency is rather low: about one marker per million phages, but this ~~easily~~ is made up for by the efficiencies of selection techniques. However, any marker is capable of being transferred, independently of the other as may be illustrated by this lantern slide,

LANTERN SLIDE 5 = fig. 5 J Bact 64:689

where the donor strain is galactose positive, xylose positive, and so forth, and the recipient is negative for these markers. If 100 million phage particles are adsorbed on an equal number of recipient cells, about 100 positive papillae can be selected on galactose agar, and the same for xylose and any other marker. But the galactose-positive cells are still xylose-negative, and vice versa. Thus, two features of the Salmonella system emerge in sharp contrast with E. coli K-12; in Salmonella, the agent of recombination is a filtrable phage particle, not the whole cell, and the unit of recombination is a small fragment, not the whole genotype. The analogy with the "transformations" of the pneumococcus and other bacteria is obvious, and suggests that these phenomena be classified together as what I have called "transductions."

The common feature of genetic transduction is that a small fragment of the total genotype is transferred. In the pneumococcus, Avery, MacLeod and McCarty could disrupt the donor bacteria by various chemical procedures, and isolate a principle plausibly, if not rigorously, shown to consist of desoxyribonucleic acid. In Salmonella, a phage particle performs this delicate operation as a by-product of its own nefarious syntheses, but while it saves us these labors, and assumes the burden of transporting the fragment and injecting it into the new host, it has so far also succeeded in denying us the access to the fragment needed for biochemical analysis.

The most plausible view of transduction seems to me to be that the fragments are indeed pieces of chromosomes, usually so short as to encompass only one marker of the several followed in any one experiment. A few exceptional cases have been found, however, which are best interpreted as the correlated transfer of two markers; these would then be factors, closely linked on the same chromosome.

Most of the markers so far studied have however shown no trace of linkage with each other.

The absence of a sexual system (at least none has yet been found) in these species has prevented the confirmation of this view by recombination

analysis; one could argue, for example, that the fragments are not just pieces of chromosomes, but whole chromosomes. This would simplify the problem of how the transduced fragment is incorporated into the new genotype, but would not readily explain how the old homologues are ejected, for which there is good evidence. It would also demand so large a number of chromosomes as to raise doubts as to the genetic stability of such a hypothetical system. However, studies are now under way on a transduction system in E. coli which seems to be limited to only a single marker, and is mechanically unrelated to sexual recombination. This promises to replace theoretical reasoning by experimental findings. I may add that some years ago and shortly before his death André Boivin had described a transduction in some strains of E. coli apparently mediated by nucleic acid (although phage is not definitely excommunicated). Regrettably, his strains have been irretrievably lost and the study of this system therefore terminated. Lacy's stuff true.

I mentioned earlier that the serological structure of the Salmonella group was one a priori indication of bacterial recombination. This premonition has been confirmed in studies with P. R. Edwards on the recombination of flagellar antigens. In most Salmonella types, these antigens

have a dual potentiality, only part of which is expressed at any one time; the oscillation is called "phase variation." By applying phage grown on one serotype to cells of another flagellar type, in the presence of homologous antiserum it is possible to select against the existing type, and recover the results of transduction of flagellar antigens. For example, phage grown on Salmonella abony, which is b: enx and applied to Salmonella typhimurium which is i: 1,2, in the presence of typhimurium serum, will evoke two new and perfectly stable serotypes, b : 1,2 and i: enx in which one phase of the recipient has been irreversibly replaced by its homologuefrom the donor. The first of these happens to be a familiar serotype, that of Salmonella paratyphi B; the second has not yet been named in the existing codification. The host range of the transducing phage permits fairly free exchange of serotypic determinants among three somatic groups of Salmonella (A, B, D) and it has therefore been possible to generate a considerable number of new combinations of the diagnostic antigens.

No final theory of phase variation has yet emerged. However, it is apparent that diphasicity represents the alternating expression of two definite unlinked loci. What determines which locus will be active and

which suppressed at any given stage is not yet known, but some indication of a local, ~~rarely reversible~~, and at least partly heritable differentiation of the ~~locus~~ genetic factor itself is given by the fact that the transducing competences of the two phases of a given serotype are at least quantitatively different. We find here a convergence of bacterial immunogenetics with developmental physiology.

The hopeful remark has been made that the geneticist will arrive too late to introduce his jargon into bacteriology--~~in fact, this was at the same meeting~~ at which the experimental results leading to the present ~~paper were first~~ published. But sophistication in biochemistry is not without its penalities either, and ~~the rewards of an indoctrination in~~ an appreciation has rewards entropy or structural organic chemistry ~~are~~ commensurate with those of selective differentials and allelomorphism linkage maps.

If the mark of scientific progress is an increasing ratio of unanswered questions, bacterial genetics scores very high indeed. But, despite the terrifying ~~though the~~ scope of the work that can be seen ahead, ~~is little short of terrifying~~, there is an underlying theme of the unity of biological processes that is indispensable in experimental design. The analogy

with the development of bacterial metabolism is a sound one, where, as C. B. van Niel has pointed out, the "unitarian approach" of comparative biochemistry has become so large a part of our thinking during the past two decades that the very fact of its having once been started is no longer taken into account." I hope the same can be said twenty years from now for comparative genetics. But as in metabolic research, we also have to beware of the fallacy that all organisms meet the common problems of biological existence by precisely the same mechanisms. But if we accept the monophyletic evolution of life, we are not surprised that these mechanisms show the stigma of family resemblance. That so many diverse organisms transfer electrons by means of phosphopyridine nucleotide is testimony of the same parallelisms as are witnessed by the universal role of recombination mechanisms and the chromosomal organization of genetic material.

The wisdom of singling out any individual for an award in science is (at best) debatable, but I am pleased to have this opportunity to acknowledge my indebtedness to my former professors, F. J. Ryan and Edward L. Tatum, to my colleagues and students already mentioned, and to someone who belongs to each of these categories, my wife.