

In the discussion, the period approximating 1930-1950 will be called "the vicennium". Its boundaries are arbitrary. When in quote-marks, "1930" will mean approximately 1928-1935; "1950" will mean 1945-1953, but before the landmark publication of the structure of DNA by Watson and Crick in 1953. We have important milestones for the vicennium: Jordan and Falk (1928) and *System of Bacteriology* (1930) at its start are magisterial reviews of prior knowledge and thought. Dubos (1945) and Burnet (1945) anticipate the modern era, and Werkman and Wilson (1951), and Gunsalus and Stanier (1962) document its early and continued progress in monographic detail. The *Annual Review of Microbiology* starting in 1947 (and several other *Annual Reviews*) and *Bacteriological Reviews*, starting 1930, offer invaluable snapshots of the contemporary state of the art. These works can be consulted for many of the pertinent bibliographic citations, and they will be explicitly repeated here only when important for the argument.

The recruitment of an active participant to write on the history of a period has some advantages, from the detail of personal recollection thus made available. The hazard of personal bias is obvious; but I hope this will be no more than an emphasis on those subjects of which I have detailed knowledge rather than any misalignment of facts or attribution of credit. There is also the bias of hindsight, which hinders a critical appreciation of the earlier state of mind; but this article will be oriented just towards an understanding of how present and future have been shaped by the scientific work of the earlier 20th Century.

Footnote: The addition x# to an attributed date indicates that the detailed reference can be found in the indicated work.

xs: *System of Bacteriology* (1930)
xd: Dubos (1945)
xj: Jordan and Falk (1928)
xg: Gunsalus & Stanier (1962)
xw: Werkman and Wilson (1951)

e.g. Kauffmann (1941xd) will point the reader to Dubos (1945) where he will find the full reference: Kauffmann, F. 1941 *Die Bakteriologie der Salmonella-Gruppe*. Copenhagen: Einar Munksgaard.

This account will center on the fundamental biology of microbes and give scant attention to continuing advances in the isolation of etiological agents of disease, and of vaccines and immunodiagnostic procedures. Most of the agents of common bacterial infections had been characterized by "1930"; but the vicennium was distinguished by important work on the classification of enteric (diarrheal) bacteria, and above all by the isolation and new study of viruses and rickettsia with methods such as cultivation virus in the chick embryo, Kilbourne (1987).

The major conceptual theme of change in microbiology during the vicennium was the convergence of the discipline with general biology. As noted by Dubos (1945),

To the biologist of the nineteenth century, bacteria appeared as the most primitive expression of cellular organization, the very limit of life. Speaking of what he considered "the smallest, and at the same time the simplest and lowest of all living forms," Ferdinand Cohn asserted: "They form the boundary line of life; beyond them, life does not exist, so far at least as our microscopic expedients reach; and these are not small." The minute dimensions of bacteria were considered by many to be incompatible with any significant morphological differentiation; it encouraged the physical chemist to treat the bacterial cell as a simple colloidal system and the biochemist to regard it as a "bag of enzymes."

Still dominated by the medical importance of microbes, the views of microbiologists in "1930" had not evolved much further, although System (1930) does have a brief chapter on bacterial cytology, and allusion to ongoing controversy over the existence of nuclear structures. Far more attention is given to the Gram Stain!

Although the diverse threads are heavily intertwined, this chapter will be organized around the following themes:

*** Be sure to reconcile ***

Nutrition, Comparative Biochemistry, and other aspects of metabolism.
 auxotrophy
 enzyme induction (adaptive enzyme formation)

Genetics Griffith - McCarty Luria & Delbruck
 Lederberg & Tatum

Evolution and Taxonomy VanNiel & Stanier; prokarya

Cell Structure

Chemotherapy (Competitive Inhibition; sulfa; antibiotics)
 receptor concept: Work & Work; Woolley

Biotechnology
 solvents; antibiotics; citric acid

Viruses, especially bacteriophage

Other microbes, including fungi; yeast; actinomycetes
 Dodge 1928 - Beadle & Tatum 1941

Miscellaneous items.

Nutrition , Comparative Biochemistry, and other aspects of metabolism.

Microbes, first yeast then bacteria, played an important part in the discovery of vitamins and other growth factors. Growth could be measured in test tubes far more expeditiously and economically than in mice, rats or humans. Conversely the realization that microbes shared virtually all of the complex growth factor requirements of animals was an important impetus to "comparative biochemistry", the view that they had a common evolution and a similar underlying architecture. One of the essential amino acids, methionine, was first discovered as a growth factor required by diphtheria bacilli (Mueller, ...), and he joined a school founded by Twort (...), and including Lwoff, Fildes, Knight and Tatum that made nutrition a branch of general biochemistry. They perceived that the requirement for a growth factor belied a loss or deficiency of synthetic power; lacking internal synthesis, the organism had to look to the nutrient environment for supply of the substance. This also implied that organisms with simple nutrition had to be empowered with complex biosynthetic capability -- leaving us humiliated by our species' inferiority to *Escherichia coli*, but that in turn is less capable than the green plant! Besides the practical utility of these findings, they led to a well founded respect for the complexity of microbial cells. In due course, especially after Beadle and Tatum (1941), the power of synthesis came to be understood as the capability of individual specific genes. That in turn led to concepts and experiments on the genetic underpinnings of metabolism.

By "1930", a number of growth factors had been shown to be important in bacterial nutrition, including factors V and X, later shown to be diphospho-pyridine-nucleotide and heme, respectively; for hemophilic bacteria; M. phlei factor, later shown to be Vitamin K, for *Mycobacterium tuberculosis*; tryptophane for *Salmonella typhi* (Fildes, 1936). Starting with the work of W.H. Peterson, H. Wood, E. Snell and E.L. Tatum at the University of Wisconsin, and of BCJG Knight and P. Fildes in England, a number of bacterial growth factors were identified with B vitamins. By "1950", most of the trace growth factors now known had been identified, and had also been associated with nutritional requirements of particular bacteria, as also had been most of the amino acids and a host of other metabolites (Snell, 1951xw). During the vicennium, most of the vitamins were also identified as co-enzymes, playing a role in the function of specific metabolic enzymes: e.g., thiamin for keto-acid decarboxylases; niacin for dehydrogenases; pyridoxal for transaminases; pantothenate in the citric acid cycle. (Schlenk, 1951xw). The 20 canonical amino acids were listed, and could be shown to be incorporated into bacterial protein.

A host of other biochemical pathways were also detailed, with the help of new methodologies of radioisotopic tracers and of chromatography. Of special significance in bacterial metabolism was the demonstration of heterotrophic assimilation of CO₂. This view of CO₂ as an anabolite was contrary to its usual image as a waste product. The specific requirements for CO₂ as a nutrient helped to clear up difficulties in the cultivation of fastidious bacteria, and eventually of tissue cells.

While a few differences in the detail of intermediary metabolism and of biosynthetic options have been discovered (e.g. for lysine), it remains true that pathways conveniently noted in

bacteria have usually been reliable predictors of the same steps in higher plants and animals. It is possible today to relate this functional conservatism to evolutionary affinity with currently available tools of DNA sequencing.

o Induced Enzyme formation -- or "Enzymatic Adaptation".

One of the most intriguing phenomena of bacterial physiology is the plasticity of enzyme expression dependent on the chemical environment. For example, *E. coli* grown on a glucose medium exhibits very low levels of B-galactosidase (lactase). When glucose is replaced by lactose, there is a growth delay followed by the abundant production of lactase. Thousands of comparable examples are now known, and the pursuit of the mechanism of this phenomenon has been of outstanding importance in the development of molecular genetics. Anecdotal reports of enzyme adaptation can be traced back to Wortmann (1882, cited in Karstrom, 1930); they were collected, together with new experimental observations by Henning Karstrom for his doctoral dissertation in Virtanen's laboratory in Helsinki. In this turning point review (Karstrom, 1930, followed by the more accessible Karstrom, 1937, and Dubos, 1940 {1945xd}) bacterial enzymes are classified as constitutive or adaptive according to their independence, or otherwise, of the cultural environment. Except for glucose metabolism, most sugar-splitting enzymes are adaptive -- resulting in substantial biosynthetic economy for a bacterium or yeast that may only rarely encounter, say, maltose now, or lactose next week. During the vicennium, the work of Stephenson and Yudkin (1936xd), and Gale (1943xd) furnished additional clearcut examples of the adaptive response, and Dubos (1945) offers a critical appraisal of the fundamental biological issues. Several theories allowed for the stabilization of pre-formed enzyme by a substrate, or a Le Chatelier-like principle of mass action to encourage enzyme synthesis. They shared the presumption that the enzyme molecule itself was the receptor of the inducing substrate. Other hypotheses lent the substrate an instructive role in shaping the specificity of the enzyme. Further progress would depend on the postulation of an enzyme-forming system distinct from the enzyme -- and this would emerge under the impetus of genetic studies to be described later. At the very end of the vicennium (Lederberg et al., 1951) described a non-inducing substrate of lactose, the analogue altrose- B-d-galactoside, which pointed to a separation of those specificities. This substrate also allowed the selection of constitutive-lactase formers, showing that lactose was not required for the conformation of the enzyme, but that the latter could be derived directly from the genetic constitution. The debate continued until the mid-1950's (See Lederberg, 1956 {P51} and Monod, 1956); it was mooted by the spectacular progress of the Pasteur Institute group in showing that enzyme induction was the neutralization of an endogenous repressor that inhibited the expression of the lactase gene in the absence of inducer (Jacob, 1965).

The simultaneous induction of several steps in a metabolic pathway, usually by an early substrate, was exploited by Stanier to delineate the later steps, notably in the oxidation of aromatic compounds by pseudomonads.

Among technical innovations, one of the most ingenious was the chemostat (Novick and Szilard, 1950). This allowed microbial populations to be maintained for the first time in a well-defined steady state, albeit under limitation for one specific nutrient.

Genetics

Interweaving themes can be dissected along ^{several} 4 planes, those culminating in

- a) Mutation studies of Luria & Delbruck (1943)
- b) Founding of biochemical genetics (nutritional mutants) of Neurospora, (Beadle and Tatum, 1941)
- c) Role of DNA in bacterial heredity -- the pneumococcus transformation (Griffith, 1928; Avery et al. 1944)
- d) Recombination in E. coli K-12 (Lederberg and Tatum, 1946)
- e) other recombinational phenomena like phage-mediated transduction in Salmonella (Zinder and Lederberg, 1942)
- f) Phage lambda, F, and other plasmids.

Bacterial genetics was substantially non-existent in 1930. As late as 1942, the eminent British biologist, Julian Huxley would suggest of bacteria that "the entire organism appears to function both as soma and germ plasm and evolution must be a matter of alteration in the reaction system as a whole." Huxley (1942). Such ideas gave little encouragement to efforts to dissect out individual genes along the Mendelian lines that had been so successful with Drosophila and other animals and plants. Some work with fungi had gotten off to a promising start early in the century (Blakeslee, 1902 utility (Rosenberg, ...). Authentic but sporadic observations of bacterial mutation, (Beijerinck, 1901xd) were outnumbered by woolly-minded speculations that embraced variations of colony form as manifestations of cellular life-cycles among the bacteria (see Dubos, 1945; Lederberg, 1990 Morange). That cloud of speculation probably discouraged more serious-minded experimentation (Zuckerman & Lederberg, 1987).

Bacteria did of course suffer from the serious methodological constraint of the apparent lack of any recombinational (sexual or crossing) mechanism by which to analyze and reconstitute gene combinations. They would prove, however, to be marvelous material for mutation studies (cf. e.g. Ames, 1975) once the concepts were clarified, for which a major turning point was the work of Luria and Delbruck, (1943). In a fashion that reminds one of Gregor Mendel, they studied bacterial mutation by quantitative counts. They used resistance to (bacterio)phage as the marker. Like resistance to antibiotics, or growth on a nutritionally deprived medium, the phage is an environmental agent that makes it easy to count exceptional cells against a preponderant background that can be selectively wiped out. Most importantly they distinguished between mutational events, which engender resistant clones, --- and mutant cells, the latter being what are counted when you plate a population with the selecting phage.

Luria, in his charming book "A slot machine, a broken test tube", (Luria, 19..) recounts how his observation of a jackpot in a gambling den inspired his premonition of the skewed statistics that would govern the numbers of mutants. The fit of experimental numbers to those statistics is subject to great theoretical uncertainty, but they were a corroboration of the clonal model. One of the first articles on bacteria to be published in Genetics, the paper promptly attracted broad attention, and was widely regarded as having proved "that bacteria have genes." The gist of the demonstration was that mutations to phage resistance agree with a clonal distribution, and thus render more likely their "preadaptive" occurrence, i.e. within the growth of the population rather than at the time of the challenge with the selective agent. It therefore harkens more to Darwin than to Mendel; but it was nevertheless a turning point in geneticists' appreciation of bacteria. The statistical methods, which are helpful in the

quantitative estimation of mutation rates, were improved by Armitage () and Coulson ().

----- Footnote

At the end of the vicennium, replica plating and indirect selection were introduced, providing constructive methods for isolating adaptive mutants: only the sibs are exposed and the clone finally isolated has never been exposed to the selective agent (Lederberg and Lederberg, 1952). From time to time there are flurries of revival of Lamarckian ideas opposed to these findings (Cairns et al., 1988). While the material basis of DNA replication offers some hypothetical opportunities for feedback from the environment to specific DNA sites, decisive experimental proof of that contingency is still lacking (Lederberg, 1989).

The themes of nutrition and mutation among microbes had occasional false starts, with observations of strain variability and the "training" of exacting bacteria to dispense with growth factors (Knight, 1936). However, lacking a conceptual framework of "genes in bacteria", these had little fruit prior to the work of Beadle and Tatum on *Neurospora* (1941). Beadle had begun his research program with Ephrussi on the genes for eye-color in *Drosophila* (Burian 1989). Tatum was engaged to do the biochemical work, but found the material almost intractable - when he approached success, he was scooped by Butenandt on the identification of kynurenine as a pigment precursor. Nor was it clear how much closer to the primary gene product this chemistry would bring them. The following account is taken from my memoir on E. L. Tatum, who was my own teacher 1946-47 (Lederberg, 1990 NAS).

This jarring experience, to have such painstaking work overtaken in so facile a fashion, impelled Beadle and Tatum to seek another organism more tractable than *Drosophila* for biochemical studies of gene action.

In Winter Quarter 1941, Tatum offered a new graduate course in comparative biochemistry. In it, he called upon his postdoctorate experience with Kogl in Utrecht, in 1937, and recounting the nutrition of yeasts and fungi, some of which exhibited well-defined blocks in vitamin biosynthesis. Beadle, attending some of these lectures, recalled the elegant work on the segregation of morphological mutant factors in *Neurospora* that he had heard from B.O. Dodge in 1932. The conjunction was that *Neurospora* had an ideal life-cycle for genetic analysis with the immediate manifestation of segregating genes in the string of ascospores. *Neurospora* also proved to be readily cultured on a well defined medium, requiring only biotin as a supplement. By February 1941, the team was X-raying *Neurospora* and seeking mutants with specific biosynthetic defects, namely nutritional requirements for exogenous growth factors.

Harvesting nutritional mutants in microorganisms in those days was painstaking hand labor: it meant examining single-spore cultures isolated from irradiated parents, one by one, for their nutritional properties. No one could have predicted how many thousands of cultures would have to be tested to discover the first mutant: isolate #299 in fact required pyridoxine. Furthermore, the trait segregated in crosses according to simple Mendelian principles, which foretold that it could in due course be mapped onto a specific chromosome of the fungus. Therewith *Neurospora* moved to center stage as an object of genetic experimentation.

In their first paper, they remarked "that there must exist orders of directness of gene control ranging from one-to-one relations to relations of great complexity." The characteristics of mutations affecting metabolic steps spoke to a direct and simple role of genes in the control of enzymes. These were therefore hypothesized to be the primary products of genes. Indeed in some cases genes might themselves be enzymes. This was an assertion of what came to be labelled the one-gene: one-enzyme theory, which has become the canonical foundation of modern molecular genetics, albeit with substantial correction and elaboration of detail, especially with regard to the intermediating role of messenger RNA, which could hardly be thought of in 1941. It would be a mistake to focus too sharply on the numerical 1:1 assertion; more important was the general assumption of simplicity, and that the details of gene expression could be learned as an outcome of such studies -- as indeed they were. (See also Horowitz, 1990).

The recruitment of *Neurospora* for what have become classical genetic studies offered further encouragement that bacteria, albeit somewhat more primitive, might be handled in similar fashion. By 1944, Gray and Tatum had produced nutritional mutants in bacteria, including some in a strain that has dominated bacterial genetics ever since, namely *E. coli* strain K-12. These mutants were shortly to be put to a most striking use.

The pneumococcus transformation. (See Lederberg, 1988 P269)

Apart from cataclysmic happenings in global war, 1944 will also be remembered for the publication of "Studies on the chemical nature of the substance inducing transformation of pneumococcal types." by Avery, MacLeod, and McCarty.

FOOTNOTE. It is awkward to have such a nondescript term as "transformation" applied to such an important, specific phenomenon. But when it was first discovered and named, there was no warrant to give it any narrower connotation. Avery had the power of new coinage but was hardly the likely personality.

This transformation / had been stumbled upon by Fred Griffith in London, in 1928, in the course of his studies on the serosystematics of pneumonia. Extracts of one serotype were evidently able to transform cells of another into the type of the first. In retrospect, it is hard to imagine any interpretation other than the transmission of a gene from one bacterial cell to another; but this interpretation was inevitably dimmed by the poor general understanding of bacterial genetics at that time.

That vagueness was compounded by two outstanding misinterpretations: 1. That the transmissible agent was the polysaccharide itself. It is sometimes overlooked that Griffith understood the distinction well enough. Better than many of his followers, he had at least the germ of a genetic theory:

" By S substance I mean that specific protein structure of the virulent pneumococcus which enables it to manufacture a specific soluble carbohydrate." and 2. That the agent was a "specific mutagen" -- e.g., Dobzhansky wrote that

"... we are dealing with authentic cases of induction of

specific mutations by specific treatments -- a feat which geneticists have vainly tried to accomplish in higher organisms." (19) This formally correct attribution, from a most influential source, obfuscates the idea that the agent is the genetic information. Muller had much greater clarity: in his 1946 Pilgrim Trust Lecture to the Royal Society, he remarked.

" ... in the Pneumococcus case the extracted "transforming agent" may really have had its genetic proteins still tightly bound to the polymerized nucleic acid; that is, there were, in effect, still viable bacterial "chromosomes" or parts of chromosomes floating free in the medium used. These might, in my opinion, have penetrated the capsuleless bacteria and in part at least taken root there, perhaps after having undergone a kind of crossing over with the chromosomes of the host. In view of the transfer of only a part of the genetic material at a time, at least in the viruses, a method appears to be provided whereby the gene constitution of these forms can be analyzed, much as in the cross-breeding test on higher organisms. However, unlike what has so far been possible in higher organisms, viable chromosome threads could also be obtained from these lower forms for in vitro observation, chemical analysis, and determination of the genetic effects of treatment. " (Muller, 1948).

Other "classical" geneticists had virtually nothing to say about Griffith's work, and would have judged themselves incompetent to assess its experimental validity. They began to pay closer attention after 1944, but again had little training in bacterial chemistry to enable them to form critical judgements about the claims presented then.

In Avery's world, however, Griffith was a central figure and his observations could not be ignored. His basic observations were confirmed in Avery's laboratory (See Dubos, 198..), and in due course Avery felt compelled to pursue the chemical extraction and identification of the substance responsible for the transformation. 16 years after Griffith, this was achieved, and DNA was thrust into the scientific consciousness as the substance of the gene.

In retrospect, it is difficult to give proper credit to the logical validity of a large range of alternative interpretations, and to reconstruct the confusions about what was meant by "gene" and "genetic". Recall that until 1951, the only marker observed in transformation was the capsular polysaccharide, the biosynthesis of which was itself subject to many conjectures, e.g. about the role of starter fragments in self-assembly. (discussed by Lederberg, 1956). Avery, undoubtedly somewhat intimidated by Dobzhansky's authority, was reluctant to put his speculations about the genetic significance of transformation in print; his famous letter to his brother, Roy, surfaced only years later. There, but not in the paper, he remarks that the "[transforming substance is] thereafter reduplicated in the daughter cells and after innumerable transfers [it] can be recovered far in excess of the amount originally used Sounds like a virus - may be a gene. But with mechanisms I am not now concerned - One step at a time - and the first is, what is the chemical nature of the transforming principle? Someone else can work out the rest." (Quoted in Dubos, 1976). As late as 1948, so distinguished a geneticist as G. W. Beadle still referred to the phenomenon as a "first success in transmuting genes in predetermined ways." (Note transmuting, not transmitting!) This obscuration of the pneumococcus transformation became less troublesome with the overall development of a bacterial genetics.

Indeed the controversy raged on the chemical claim, that the substance was DNA (and nothing else!). {This story is detailed by Judson () and in McCarty's personal memoir (1987).} Alfred Mirsky, Avery's colleague at the Rockefeller Institute, was a vocal critic of the chemical identification of the transforming agent. I believe he was quite persuaded that this was an instance of gene transfer, but the more reluctant to concede that the evidence to date settled so important a question as the chemical identity of the gene as pure DNA (versus a complex nucleoprotein). Avery himself had cause to worry -- there had been much resistance to his earlier proofs that pneumococcal polysaccharides, free of protein, were immunogenic. Wendell Stanley's first claims that crystalline tobacco mosaic virus were pure protein had to be subject to humiliating correction when ribonucleic acid was also found therein. We should recall that when most biologists of that era used terms like protein, nucleic acid, or nucleoprotein, it can hardly be assumed that they had today's crisp connotations of defined chemical structure. These issues could only be settled by the few experts who had worked with these materials experimentally -- and it was a daunting task to prove that there were too few molecules of any contaminating protein in the "DNA" to account for its genetic specificity. Maclyn McCarty's meticulous work continued to provide ever more persuasive evidence that it was DNA, and the contemporaneous studies of Chargaff, showed that DNA was far more complex than Levene had figured it to be, and therefore capable of the subtlety demanded of a "gene". Rigorous proof about "DNA alone" was really not furnished prior to the production of genetically active synthetic DNA three decades later. By 1952, Hershey and Chase gave evidence from an independent quarter that DNA alone penetrated the phage-infected cell. This was not quantitatively certain; but by then most of the critics had exhausted themselves. In the following year, the structural models of DNA as a double helix (Watson and Crick, 1953) lent final plausibility to "DNA alone."

This episode is sometimes painted as unreasonable resistance to a new idea (Stent, 19). That is hardly a fair assessment of a controversy that was settled within 9 years, and which required the emergence of a new class of workers, and conversion of some of the old ones, to deal with new techniques and experimental materials. That there was continued controversy is appropriate to the spirit of scientific skepticism -- more to worry about when challenging new ideas are merely ignored.

Genetic recombination in bacteria. (See Lederberg 1987 {P269})

All three of the foregoing threads -- selected clonal mutation; Neurospora biochemical genetics and the penumococcus transformation were important instigations, but the last was the most immediate impulse to inquiry about genetic recombination in bacteria. Whatever may have been the immediate reaction to Avery et al. (1944), it electrified the Department of Zoology at Columbia University. I was a medical student working in the laboratory of Francis J. Ryan, initially on Neurospora -- he had been the first postdoctoral fellow to join Beadle and Tatum after 1941. Alfred Mirsky was a frequent visitor from the Rockefeller, and kept us up to date -- despite his criticisms of the chemistry. Whether the transforming factor was pure DNA was of course important, but there was a certain inevitability to the correct resolution of that controversy. Equally important: was the transforming factor really a gene, a unit of Mendelian heredity? How could we verify that, when one knew nothing else of the genetics of bacteria? Unless that were settled, the chemical controversy would center on a

curiosity, not the central issue of contemporary biology.

Under Ryan's guidance, I first tried to transform *Neurospora* with DNA containing extracts. This was unsuccessful (and would be in others' hands for three decades). The paradigm combined the ideas of stringently selected mutation (Luria and Delbruck) with the use of auxotrophic, growth-factor dependent, mutants (Beadle and Tatum). It did demonstrate the occurrence of spontaneous reverse mutations to prototrophy (growth factor independence), But to ring the changes, if one couldn't transform *Neurospora*, an alternative was to explore the genetics of easily handled bacteria like *E. coli*. The pneumococcus was a dangerous pathogen; and the Avery group's accounts were full of premonition about the difficulty of getting reproducible results with it. The organism was difficult to grow, and no other selectable markers were then available besides the serotype.

After a few preliminary experiments at Columbia, Ryan suggested I apply to work with Tatum, who in Fall 1945 was just moving to Yale; and I would be released from naval service (in training) with the conclusion of World War II. While I had isolated some others, the mutants reported by Gray and Tatum (1944) would be immediately helpful. (I could not know that more importantly, K-12 was a serendipitously marvelous choice of strain of *E. coli*).

Tatum very graciously invited me to join him in New Haven, which I did in March 1946. My first actual crossing experiments entailed the selective isolation of prototrophs from (conjugating) mixtures of complementary auxotrophic stocks. They were promptly successful. After a dozen replications, the work was ready to be presented at the Cold Spring Symposium in July. We were fortunate to be able to get the first confrontation in front of nearly every expert in the world all at once, under the discipline of their mutual critical outlook. In short order, a dozen other genetic markers were incorporated into the studies, and it was possible to generate the first linear linkage maps (Lederberg, 1988). One of the first investigators to corroborate the reports was Luca Cavalli-Sforza, then a fellow with R. A. Fisher at Cambridge. There, he was the first to find crossable strains other than K-12 (Cavalli and Heslot, 1949); at the end of the vicennium, and after his return to Milan, his discovery of hyperfertile (Hfr) and self-sterile (F-) strains would furnish the key to the elucidation of sexual mechanisms during the following decade. (Cavalli-Sorza et al. 1953; Hayes, 1953-CSH; Hayes 1964; Jacob and Wollman.).

Why did crossing in bacteria take until 1946? The central experiment is so simple that it is readily done in high school. Assertions of contrafactual history can never be verified; but the myth of bacterial asexuality must be given some credit (Zuckerman and Lederberg, 1987). Microbiology began with the first microscopic visualization of microbes under the hands and eyes of Leeuwenhook in 1676. In 1695 he reported on a wide variety of animalcules, larger than the tiny dancing dots, the bacteria. These larger animals were the protozoa, which exhibited overt copulation. That protozoa were sexual, while bacteria were not, was then an attribution of the *Scala Naturae* that was firmly engrained with the very initiation of microbiology and reaffirmed with Koch's pure culture methodology. (Cf. Dubos, 1945). This concept of bacteria as organisms that breed true to type and divide only by fission became crystalized in 1875 in the first formal taxonomy of bacteria. Ferdinand Cohn called them the Schizomycetes, the "Fission Fungi". The myth of bacterial asexuality was thus engrained in

the very class names of the organisms in question.

With new confidence that bacteria were programmed by genes, many investigators began to use them for studies of chemical mutagenesis, notably the school of M. Demerec at Cold Spring Harbor (Demerec and Hanson, 1953). The selection paradigm allows billions of cells to be tested on one plate, and the results can be read overnight. It became quickly possible to corroborate that hundreds of chemical reactive substances, many already inculpated by other experiments, were mutagenic -- including, for example, nitrogen mustard. Many other common substances like formaldehyde, organic peroxides, and manganous ion also became suspect. (Cf. Lederberg et al. 1951). The public health significance of these findings was not, however, widely appreciated before the 1960's; it was then accentuated by the development and popularity of the Ames test, {1975} which uses the same procedures in a standardized way. The very simplicity of these procedures demands cautions in interpretation: many artefacts have been encountered where differential (selective) killing of cells has been confused with mutagenesis (Lederberg 1948). The most lethal and reactive chemicals may be the least concern as mutagens, the lethality being the overriding effect. Very reactive substances, like formaldehyde, may have difficulty reaching vital cellular targets intact; but reversible complexes like Schiff bases for formaldehyde and chloramines for chlorine may then be the most insidious intermediaries. The nastiest mutagens, like the nitrosoguanidines, are those with lowest acute cell lethality.

Even during the vicennium, the mutational theory of cancer was at least visible, if not widely preferred; as with radiation, it was predicted that mutagens would also be carcinogenic (e.g. Tatum,) which would dominate the environmental health concerns about these chemicals.

Chemotherapy; antibiotics and drug-resistance; competitive inhibition. (Dubos 1945; Albert 1985; Work and Work, 1948).

Quite independently of the turning points in genetic doctrine, the vicennium was also the interval of development of the antibiotics and other chemotherapeutic agents that ushered in modern pharmaceuticals. These in turn had disparate routes.

Antibiotics (cf Waksman, MacFarlane, Wilson) had a spectacular beginning with the famous discovery of penicillin by Fleming in 1928, a mold spore having accidentally lodged on agar plates seeded with staphylococci. The isolation of the antibiotic from the crude culture filtrates was a formidable chemical task, but was undertaken successfully in the late 1930s by Florey and Chain in England, and followed by the industrial production of penicillin as a joint US-British war project. For this to be feasible required a substantial effort in strain improvement, which was conducted, however, along empirical rather than rational genetic lines, (Wilson). This was nevertheless the forerunner of the modern fermentation industry and biotechnology; its antecedents had been the production of butanol and acetone as munitions solvents during World War I, and the peacetime production of citric acid by a mold fermentation.

Meanwhile, S. Waksman and R. J. Dubos had been studying the biochemical and ecological interrelations of soil microbes. The role of secreted antibiotics in ecological competition provided a rationale for seeking these substances. Tyrothricin (Dubos 1939, cf. Crease, 1989)

was the first antibiotic to be clinically applicable but its systemic toxicity limited its application to topical treatment. In Waksman's hands, the same paradigm led to the discovery of streptomycin, (1944), and thereafter a continued stream of new antibiotics having untold human benefit. It would be some time before the mode of action of antibiotics would even begin to be understood (Cf. Gottlieb and Shaw, 1967) and to allow rational principles to assist in their improvement.

The chemotherapeutic revolution began, even prior to these antibiotics, with the introduction of sulfanilamide, a simple synthetic compound, in 1936. Although used when nothing else was available, Paul Ehrlich's arsenical "magic bullets" (like salvarsan, 1909xd) were so toxic as to discourage the hope of finding substances with useful selective toxicity against microbes, (Albert 1951, 1985; Work and Work, 1948). Ehrlich did lodge the idea of the receptor, "Corpora non agunt nisi fixata", that drugs could only act if they were bound to the target organism. This led to continued studies on dyestuffs which had visible affinities for microbes -- though we now know that their color is immaterial. In 1935, such a dye, Prontosil, was introduced for the treatment of streptococcal infection, (Domagk, 1935, NP). Its spectacular efficacy was followed by the metabolic studies which showed that the active unit was sulfanilamide, released from its azo (dye) linkage in the body. Hundreds of sulfanilamide analogues, collectively the sulfonamides, have since been prepared and retain a permanent place in the pharmacopeia. Sulfanilamide is antagonized by nutrient media, blood, and bacterial culture filtrates, an observation that led to the identification of PABA (p-aminobenzoic acid) as a specific antagonist, and the speculation that PABA is an essential metabolite. This was later confirmed when it was found to be required by various bacteria and some *Neurospora* mutants. Woods' (1940) and Fildes' (1940) concept of competitive inhibition by structural analogues of essential metabolites has been the foundation of rational therapeutics for the rest of the century, of which the development of AZT for the treatment of AIDS is only the most recent example. (Hitchings, 1988).

Cell Structure. #s. (Spooner and Stocker, 1956; Brock, 1988)

Sophisticated studies of bacterial cell structure began only at the end of the vicennium, with the introduction of new methods such as electron microscopy and immunofluorescent staining with specific antibodies. Descriptions of bacterial chromosomes and nuclei (I charitably avoid specific references) did not survive the new techniques. However, several workers had demonstrated Feulgen-staining regions in bacteria, the localization of the DNA which could be chemically extracted. This was corroborated by the use of deoxyribonuclease to remove that basophilic staining material, (Tulasne, 1953xs). The light microscope simply does not provide sufficient resolution to scrutinize the details. Bacterial genetics therefore lacked the bolstering from cytology that had assisted in the development of genetic / chromosome theory in other organisms.

Evolution and Taxonomy. Stanier, RY and van Niel, CB 1962 Stanier, RY 1964

These problems of cell structure also impeded the development of a rational taxonomic definition of the bacteria, long classified as primitive fungi. DNA sequence similarity, the gold standard of phylogenetic affinity, was also unavailable during the vicennium. The discussions of these matters have become almost unintelligible in retrospect, as remarked by

some of the protagonists themselves. The species nomenclature has been equally arbitrary - for example, virtually all distinguishable serotypes of *Salmonella* have been given binomial names, like *Salmonella typhimurium*, *S. pullorum*, *S. newport* -- the easy production of new serotypic combinations by genetic transduction (Iino World Salm Problem) notwithstanding. Nevertheless, this "splitting" has had the heuristic value of encouraging the publication and compilation of new serotypes, and the establishment of an epidemiologically valuable system (Kauffmann, 1945xd) for the *Salmonella* group. The definition of "species" in largely clonal organisms follows no simple paradigm, like that of the shared gene pool in strictly sexual forms. Even today, we are just at the start of empirical studies of the diversity of clones of various bacteria as they occur in nature.

Fungi; yeast; actinomycetes: (Catcheside, 1951)

Fungal genetics was given a great boost during the vicennium by the *Neurospora* example. The taxonomy and physiology of the actinomycetes, if not yet their genetics, gained a special focus in the light of their newly manifest role in the soil, and their industrial importance in the recovery of antibiotics (Waksman). Winge and Laustsen, (1937) at the institute funded by the Carlsberg Brewery, initiated yeast genetics with controlled crosses that revealed heterothallism in ascosporegenous strains, and eventually involved mendelizing markers similar to those used in *Neurospora*. Yeast genetics during the vicennium was, however, confounded by imputations of "plasmagenes", self-reproducing particles supposed to emanate from the nucleus but retain their autonomy in the cytoplasm. (Spiegelman, 1946 CSH). In retrospect, irregular gene segregation and gene conversion should be invoked to account for the observations of that school. On the other hand, the "petite colonie" variant of yeast has provided a striking example of cytoplasmic inheritance -- the trait being induced in a large proportion of cells exposed to acriflavine (Ephrussi et al., 1949 AIP). This system is still DNA-based - the cytoplasmic particles being now identified with the mitochondria. While the importance of these extranuclear elements is beyond controversy, they lend little support for the idea that the cytoplasm as an integrated network matches the nucleus in providing genetic information (Sapp,).

Viruses and lysogeny. The plasmid concept; Luria 1953 textbook. Mark Adams 19); Hayes 1964; Galpern 1988. Burnet (1945)

Biology of the virus

The cardinal discovery for virology was the isolation and crystallization of the tobacco mosaic virus (Stanley, 1935), which sharpened many questions about this boundary of living existence (Pirie - Meaninglessness ...). A more convenient system for virus biology proved, however, to be the viruses attacking bacterial hosts, the (bacterio)phages, especially in the hands of the Delbruck school (Adams, 1959). Their life cycle was worked out in some detail, eventually culminating in two cardinal experiments:

Hershey and Chase (1952) - the DNA of the attacking phage particle is sufficient to initiate infection. The DNA (not the entire phage) replicates in the host bacterium, then generates the capsid and assembles itself into mature, infectious phage particles.

Hershey (1946 CSH) different phage genomes can undergo genetic recombination, enabling

the construction of linkage maps. These would eventually be constructed in ultimate detail, matching the DNA sequence of the nucleotides.

Viruses were defined by Luria (1953) as "sub-microscopic entities, capable of being introduced into specific living cells and of reproducing inside such cells only." He pointed out that this is a methodological rather than taxonomic criterion; such a definition might well embrace a wide range of diverse entities. By 1950, he had been able to insist that the phages exhibited "parasitism at the genetic level", taking over the metabolic direction of the host cell, and exploiting a wide repertoire of its genetic capabilities. It would remain to be seen whether other viruses, in plant and animal cells, would share these attributes.

Lysogeny.

Not long after the Twort-d'Herelle discovery of the bacteriophages (1915-1917), bacterial cultures were found that appeared to have established a durable symbiosis with a resident phage. The Delbruck school tended to dismiss these as contaminants, despite persuasive arguments, i.a. of Burnet and Lush (1936). Lwoff and Gutmann (1950) reentered the controversy and showed that lysogenic Bacilli carried a "prophage", a genetic capability of producing the phage. At the same time, Lederberg and Lederberg (1951, 1953) had discovered that *E. coli* K-12 was lysogenic, for a phage they named "lambda", as a parallel (or so they thought) for the kappa particles in *Paramecium*. Crosses of lysogenic with sensitive strains however showed that the capacity to produce lambda segregated in close linkage with a chromosomal marker (*gal*); and they therefore invoked Lwoff's concept and terminology of prophage. However, the working out of that story, and of the phenomena of phage-mediate transduction belongs to the next era.

All these discoveries, taken together, gave substance to Luria's vision of the virus as a genetic element that is coordinated with the genome of the host, but with pathogenetic consequence that has evolved to suit the needs of the parasite. The host may also co-evolve to reach an equilibrium compatible with the survival of both partners -- a general principle in the evolution of pathogenicity (Th. Smith, 1934).

Prospects of cytoplasmic heredity fascinated many workers, even during the working out of the nuclear (Mendelian) basis of microbial biology, perhaps as a carryover of Huxley's idea of the persistent soma. In the course of the discussion, there were angry ripostes as to whether a given entity was really a plasmagene, or perhaps a virus, or perhaps a symbiont. The term and concept "plasmid" was introduced (1952) to stress the operational vacuity of those distinctions. A particle could be at the same time a virus (if one focusses on pathology), or symbiont, or plasmagene (if one focusses on the genetic role). As a prophage it may even be integrated into the chromosome, with a potential reappearance later. And it would be impossible to say whether a virus had evolved its pathogenicity, having once been a benign organelle, or vice versa, or both at different evolutionary epochs. One might even revive Altmann's old picture of the mitochondria as originally symbiotic bacteria, an allusion founded merely on the limitations of cytological analysis.

The vicennium worked a transformation, the "biologization" of the microbe. It was an extraordinarily exciting and fertile time, with new phenomena to be found in every culture

dish. One could even learn to treasure one's contaminations.