tic recombination in bacteria (by the protocol that later proved to be successful). These notes coincide with the beginning of my course in medical bacteriology. They were provoked by the contrast of the traditional teaching that bacteria were *Schizomycetes*, asexual primitive plants, with an appreciation of sexuality in yeast[6], which was represented at Columbia by the graduate research work of Sol Spiegelman and Harriett Taylor.

Dubos[5] cited many unclear, and two clear-cut negative results[7,8] for sexuality in bacteria using genetic exchange methodology. But these two studies had no selective method for the detection of recombination and so would have overlooked the process had it occurred less often than perhaps once per thousand cells. With the use of a pair of nutritional mutants, say A^+B^- and A^-B^+, one could plate out innumerable cells in a selective medium and find a single A^+B^+ recombinant. In early July, I began experiments along these lines. In the first instance I used a set of biochemical mutants in *Escherichia coli*, which I began to accumulate in Ryan's laboratory. To avoid the difficulty that had arisen in our *Neurospora* experiments, a spontaneous reversion from A^-B^+ to A^+B^+, the strategy would be to use a pair of double mutants: $A^-B^-C^+D^+$ and $A^+B^+C^-D^-$. Sexual crossing should still generate $A^+B^+C^+D^+$ prototroph recombinants. These would be unlikely to arise by spontaneous reversions which, in theory, requires the coincidence of two rare events; $A^- \rightarrow A^+$ and $B^- \rightarrow B^+$. Much effort was devoted to control experiments to show that double reversions would follow this model, and so occur at a negligible frequency in the cultures handled separately. Thus the occurrence of prototrophs in the mixed cultures would be presumptive evidence of genetic recombination.

Long shot

Meanwhile at Stanford, Ed Tatum, whose doctoral training at Wisconsin had been in the biochemistry of bacteria, was returning to bacteria as experimental subjects, having published two papers on the production of biochemical mutants in *E. coli*[9], including double mutants like those described here. During the summer of 1945 Francis Ryan learned that Tatum was leaving Stanford to set up a new programme in microbiology at Yale. He suggested that, rather than merely ask Tatum to share these new strains, I apply to work with him and get the further benefit of his detailed experience and general wisdom. Tatum agreed and suggested that I arrive in New Haven in late March, to give him time to set up his laboratory. He hinted that he had some similar ideas of his own, but never elaborated them. The arrangement suited him by leaving him free to complete his work on the biochemistry of *Neurospora*, perform the heavy administrative duties of his new programme, and still participate in the long-shot gamble of looking for bacterial sex.

It took about six weeks, from the first serious efforts at crossing in mid-April 1946, to establish well-controlled, positive results. These experiments could be done overnight, so the month of June allowed over a dozen repetitions, and the recruitment of almost a dozen genetic markers in different crosses. Besides the appearance of $A^+B^+C^+D^+$ prototrophs, it was important to show that additional unselected markers in the parent stocks would segregate and recombine freely in the prototrophic progeny. This result left little doubt as to the interpretation of the experiments.

An immediate opportunity for public announcement presented itself at the international Cold Spring Harbor Symposium in July. This was dedicated to the genetics of microorganisms, signalling the postwar resumption of major research in a field that had been invigorated by the new discoveries with *Neurospora*, phage, and the role of DNA in the *Pneumococcus* transformation. Tatum was already scheduled to talk about his work on *Neurospora*. We were granted a last-minute improvisation in the schedule to permit a brief discussion of our new results.

The discussion was lively. The most principled criticism came from Andre Lwoff who worried about cross-feeding of nutrients between the two strains without their having in fact exchanged genetic information. Having taken great pains to control this possibility, I felt that the indirect genetic evidence was quite conclusive. Fortunately, Max Zelle mediated the debate, and generously offered to advise and assist me in the direct isolation of single cells under the microscope. These subsequent observations did quiet remaining concerns of the group that Lwoff had assembled at the Pasteur Institute, including Jacques Monod, Francois Jacob and Elie Wollman, who were to make the most extraordinary contributions to the further development of the field. The single cell methods were also useful in later investigations in several fields. A direct result of the Cold Spring Harbor meeting was the prompt ventilation of all the controversial issues. With a few understandable, but minor points of resistance, genetic recombination in bacteria was soon incorporated into the mainstream of the burgeoning research in molecular biology, and after another decade or so into the standard texts of bacteriology. It still took some years to work out the intimate details of crossing in *E. coli*; some, including the crucial question of the physical mechanism of DNA transfer between mating cells, are still obscure.

The public image of the scientific fraternity today has seldom been so problematic and the system cannot avoid putting a high premium on competition and self-assertion. We can recall with gratification how the personalities of Ryan[10] and Tatum[11] exemplified norms of nurture, dignity, respect for others, and above all a regard for the advance of knowledge.

Experimental luck

1. We have learned[12] that *E. coli* strain K–12 itself was a remarkably lucky choice of experimental material: only about one in twenty randomly chosen strains of *E. coli* would have given positive results in experiments designed according to our protocols. In particular, strain B, which has become the standard material for work on bacteriophage, would have been stubbornly unfruitful. Tatum had acquired K–12 from the routine stock culture collection in Stanford's microbiology department when he sought an *E. coli* strain to use as a source of tryptophanase in work on tryptophan synthesis in *Neurospora*[13]. The same strain was then in hand when he set out to make single, and then double mutants in *E. coli*[9]. In 1946, I was very much aware of strain specificities and was speculating about mating types (as in *Neurospora*). I have no way to say how many other strains would have been tried, or in how many combinations, had the June 1946 experiments not been successful.

2. An equally important piece of luck was that, the selected markers Thr (threonine) and Leu (leucine) are found almost at the origin of the *E. coli* chromosome map[14]. The cognoscenti will recognize that in a cross $B^-M^-T^+L^+F^+ \times B^+M^+T^-L^-F^-$, the configuration used in June 1946, these chromosome localizations offer almost a maximum yield of selectible recombinants. We were therefore led stepwise into the complexities of mapping.

Joshua Lederberg is at the Rockefeller University, New York, New York 10021. *The research summarized in this article was supported in 1946 by a fellowship of the Jane Coffin Childs Fund for Medical Research.*

1. Lederberg, J. & Tatum, E.L. *Nature* **158**, 558 (1946).
2. Beadle, G.W. & Tatum, E.L. *Proc. natn. Acad. Sci. U.S.A.* **27**, 499 – 506 (1941).
3. Avery, O.T., MacLeod, C.M. & McCarty, M. *J. exp. Med.* **79**, 137 – 158 (1944).
4. Ryan, F.J. & Lederberg, J. *Proc. natn. Acad. Sci. U.S.A.* **32**, 163–173 (1946).
5. Dubos, R. *The Bacterial Cell* (Harvard, Cambridge, 1945).
6. Winge & Lausten, O. *C.R. Lab. Carlsberg, Ser. physiol.*, **22**, 99-119, (1937).
7. Sherman, J.M. & Wing, H.U. *J. Bact.* **33**, 315 – 321 (1937).
8. Gowen, J.W. & Lincoln, R.E. *J. Bact.* **44**, 551 – 554 (1942).
9. Gray C.H. & Tatum, E.L. *Proc. natn. Acad. Sci. U.S.A.* **30**, 404–410 (1944).
10. Lederberg, J. *in University on the Heights*, (ed. First, W.) 105 – 109. (Doubleday, Garden City, New York, 1969).
11. Lederberg, J. *A. Rev. Genet.* **13**, 1–5 (1979).
12. Lederberg J. *Science* **114**, 68 – 69 (1951).
13. Tatum, E.L. & Bonner, D.M. *Proc. natn. Acad. Sci. U.S.A.* **30**, 30 – 37 (1944).
14. Bachmann, B.J. *Microb. Revs.* **47**, 180 – 230 (1983).