# Charlotte Auerbach (1899–1994)



CHARLOTTE AUERBACH, "LOTTE" to all who knew her, was born in Krefeld, Germany, in 1899. She died in Edinburgh in March, 1994. She was born into a family that was already noted for its contributions to science. Her grandfather was credited with the discovery in 1862 of "Auerbach's plexus," collections of nerve fibers within the walls of the intestine supplying its muscle layers and controlling its peristaltic movements. One of the stories LOTTE enjoyed telling about him emphasized his absorbing interest in science. It relates to events on his honeymoon in Würzburg. On arriving there he left his new wife in the hotel while he went in search of a scientific colleague, a professor of anatomy and physiology. The discussion that followed was so absorbing that her grandfather forgot his bride and happily accepted a dinner invitation from the professor for that evening. Later he remembered why he was really in Würzburg and returned hurriedly to the hotel, only to find his wife packing to return to mother. Fortunately, this forgetfulness was forgiven and the marriage was a happy one.

LOTTE's father was a physical chemist who, himself, gained some distinction, graduating in Breslau in 1893 and working first in the chemical industry and later in the Ministry of Health in Berlin. In the latter position he became sufficiently senior to have graduate students. He also did research on poisons, formaldehyde, and other agents. During this time he was initially co-editor with ABEGG of the *Handbuch der anorganischen Chemie* and later became its sole editor. LOTTE's mother, SELMA, was the daughter of a general practitioner in Jauer, Silesia. She loved poetry, literature and music and apparently had a very lively mind.

LOTTE was thus born into a cultured and intellectual Jewish family on the 14th of May, 1899. A few years later her family moved to Berlin where she was to grow up. Her interest in biology was kindled by her father who took time to teach her to identify birds and plants as well as the constellations in the night sky. Indeed, I remember not so long after we arrived in Edinburgh being invited to a very cold star-gazing party at Upper Gray Street when she proudly demonstrated her new telescope which stood mounted on its tripod in the back yard of her house. Natural history was one of her father's passions and he shared it with her, encouraging her curiosity and feeding her intelligence. Her interest in biology was not fostered in school, however, and she received no formal instruction in the subject after she was 15. In spite of this she recalled an extracurricular lecture on the behavior of the chromosomes at cell division which she heard at secondary school and which she described as one of her few truly spiritual experiences. She suddenly saw the field of biology opening up before her and decided there and then to study it.

Her undergraduate years were spent, as was possible in Germany, working in the Universities of Würzburg, Freiburg and, finally, Berlin. Among her teachers during this period were KNIEP, SCHLEIP and SPEMANN. In 1924 she passed her Staatsexamen in biology, chemistry and physics, but she doubted whether she could be

original and independent enough to become a good scientist and decided to train as a secondary school teacher. In addition to her doubts about her own abilities, she also was very fond of children and was anxious to maintain human contacts in her work. She also realized that a Jewish woman without private means would stand little chance of a career in a German university. So, in 1924 she completed her training as a teacher and for a short time she taught in Heidelberg and Frankfurt.

This period was not very happy and it was apparent that she suffered greatly from the anti-Semitism of the other members of the staff and from some of the children. This ended in 1925 when she came into a small legacy and started work on a Ph.D degree in Berlin-Dahlen at the Kaiser-Wilhelm-Institut für Biologie under MANGOLD, but she was unhappy with her project. When she suggested to her professor that she would prefer to change direction, he said, "You are my student, you do as I say. What you think is of no consequence!" LOTTE was not one to compromise in such a situation and, assessing her prospects in the University as unpromising, she left to take up secondary school teaching, this time in Berlin.

In view of her later success as a teacher of undergraduates, it is perhaps surprising that she did not find teaching easy. Keeping order in class exhausted her, and the increase in anti-Semitism that permeated society at that time probably also conspired against her. It may have been a blessing in disguise, therefore, when in 1933 all Jewish secondary school teachers were summarily dismissed and she was forced again to think about her future. She took her mother's advice, left Germany and, with the help of family friends in London and Edinburgh, was able to join the University of Edinburgh and to complete her Ph.D at the Institute of Animal Genetics. She received the degree in 1935 in spite of initial difficulties that arose because of incompatibilities between the German and the British systems of education and because she had misunderstood the University regulations. At first her degree was refused but it emerged that this was because she had only submitted two thesis copies, one finished and the other rough, which she had assumed was simply for the records. The latter had been sent to the examiner. With the help of PIO KOLLER, who was at the Institute and was able to make enquiries of the examiners, the whole thing was straightened out and the degree conferred. The subject was the development of the legs of Drosophila.

The next few years were difficult. LOTTE became personal assistant to CREW. This involved cleaning cages of mice and budgerigars, which she quite naturally resented, but she also became involved in other activities such as translation, teaching, and some research into Mendelian genetics. The main compensation, however, was being in contact with the very lively group of workers which CREW had assembled at the Institute, a number of whom were, like LOTTE, refugees from totalitarian European regimes. Among the people she frequently spoke of from that time were GUIDO PONTECORVO, PIO KOLLER and, later, the SLIZYNSKIS (BRONISLAW and HELENA), who were closely involved with some of LOTTE's later work well into the 1960s. In addition to these more permanent members, CREW also managed to attract distinguished names to give lectures or to work for a few weeks. These include JULIAN HUXLEY, J. B. S. HALDANE and, most importantly from LOTTE's standpoint, H. J. MULLER. These were pivotal years for her. She became a British citizen in 1939, and this helped her to continue with her scientific career unimpeded while many other foreign scholars were interned. In fact, she records that, in the same post that brought the news of her new nationality, came also the letter requiring that she register as an alien. Two weeks before the outbreak of war in 1939 her mother joined her from Germany. (Her father had died of a heart attack in 1925.) LOTTE's position in the Institute was ill-defined and very poorly paid, but the realization of what they had escaped in Nazi Germany was compensation for the lean times they experienced at the outset of the 1940s. And there were other compensations. She recalls that HANS GAL, the distinguished Austrian composer, formed an orchestra from a group of German and Austrian refugees in which LOTTE played the cello.

However, the person who influenced LOTTE's subsequent scientific development most profoundly was HERMAN MULLER. He came to the Institute in 1938 and remained until 1940. CREW told MULLER in front of LOTTE that she would do cytological work for him. This drew a quick refusal from her, "No, I'm sorry. I'm no good at cytology." CREW told her that as his assistant she should do what he said, and went away. MULLER, however, assured her that he only wanted people to work on his projects who were interested in them and later asked her what were her interests. She replied that she was interested in gene action in development. MULLER then reminded her that if she wanted to understand the gene, it would be important first to understand what happens when it mutates. This eventually led to a discussion of substances that might react with the gene material and cause it to mutate, and these exchanges were to set the course of her long career.

MULLER's inspiration and the observations of an Edinburgh pharmacologist, A. J. CLARK, on the similarities between the pharmacology of X rays and mustard gas led LOTTE to test the ability of mustard gas to cause mutations. CLARK had noted that the lesions produced by mustard gas were slow to heal and tended to reappear, as is the case for somatic lesions produced by X-rays. MULLER had already reported the mutagenic action of X rays, and the similarities between the lesions caused by the two types of agent suggested that the induction of mutations might also be part of the action of mustard gas. In collaboration with J. M. ROBSON,

who had also noted this similarity, and using MULLER's elegant genetic system to detect sex-linked recessive lethals, a clear positive effect was obtained almost at once. In the first experiment, which was based on approximately the same number (>1200) of treated and untreated male chromosomes, the percentage of lethals in the control was 0.25, whereas in the treated chromosomes it was 7.7. In a later experiment the figure was 8.6% in 790 treated chromosomes. Other agents were also tested at this time. Vesicants such as Lewisite and osmic acid proved to be ineffective as mutagens, and ammonia, though possibly having a weak action, failed to produce significant increases. Because the War had started by this time and it was feared that mustard gas might be used against Britain, the results of these experiments could not be published until 1946. The reports were retained by the Ministry of Supply. Even this delay had its advantages, because LOTTE was able to conduct an unhurried and careful comparison of the genetic effects of alkylating agents and ionizing radiation. By the time publication was permitted she had an impressive corpus of observations to present to the scientific community, and on the basis of this work she was awarded the prestigious degree of DSc. from the University of Edinburgh in 1947 and appointed a University Lecturer. In 1949 she was elected to fellowship of the Royal Society of Edinburgh and in 1958 to fellowship of the Royal Society of London. In 1958 the University made her Reader. Her personal chair followed in 1967.

Her comparisons between the mutagenic effects of ionizing radiations and chemicals in Drosophila occupied much of the next twenty years and formed the basis for her research interests to the end of her working life. From the very first experiments it became clear that chemically produced mutations, whether visibles or sex-linked recessive lethals, were predominantly mosaics of mutant and non-mutant cells, whereas those induced by ionizing radiation were whole-body changes. Furthermore, the ratio of point mutations to chromosome mutations was different, being much higher after chemical treatment than after ionizing radiation. Her thorough knowledge of Drosophila biology was put to use to investigate this difference in a study of the effects of storing sperm from treated males in the seminal receptacles of the untreated females with which they were mated. Fertilization takes place only when the female is encouraged to lay her eggs, and egg laying can be delayed for several days under appropriate conditions of culture. These studies showed that during sperm storage there was an increase in the frequency of chemically induced chromosome mutations that was not matched by an equivalent increase in point mutations and, as a result, the spectrum of mutations induced by alkylating agents gradually approached that of X rays. This finding led to the proposal that alkylating agents produce "latent" chromosome breaks which, in contrast to those produced by ionizing radiation, take time to open and rearrange. Storage of the treated sperm increased the probability of this happening before fertilization and subsequent cell division and hence made it more likely that chromosome rearrangements would affect both daughters of the dividing zygote and not be lost as mosaics. Careful cytological studies by SLIZYNSKA (see above) tested this idea: in a detailed study of small deficiencies, she was able not only to produce evidence in support the interpretation but also to demonstrate that potential breaks within the same chromosome tended to mature together, with the result that intrachromosomal rearrangements were more frequent than interchromosomal exchanges. The nature of the lesions and the mechanism of the delayed effect was, however, never elucidated. There was much excitement over the elegant chemical studies of PETER BROOKES and PHIL LAWLEY on the alkylation of DNA and its enhanced and extended depurination, but even these findings failed to provide a satisfactory and, more important, easily testable solution to the problem.

Another aspect of the delayed effects associated with chemical mutagens that fascinated LOTTE came from the observation that chemical mutagens appeared to induce a gene state which could only be described as unstable. The crucial points about these lesions, which she took great pains to point out, were that (a) their inheritance was not unilineal—they were clearly able to replicate as unstable lesions so that both daughter cells and not only one of them received a copy of the unstable gene from the mother cell, and (b) the instability continually generated stable mutations that appeared to be allelic using the rather crude genetic criteria that were then available. What was also puzzling was that the lesions were apparently produced mainly in response to mutagenic treatments, which at the time made explanations based on transposing elements less attractive. Replicating instabilities were demonstrated for sex-linked lethal mutations in Drosophila, recessive lethals in Neurospora, and mutations to adenine auxotrophy in yeast cells. In the last case the consequences of instability were readily manifested as color-sectored colonies. However, ensuring that these were not an artifact caused by the clumping of mutant and non-mutant cells required great ingenuity and occupied the working hours of many associates and visitors to the lab. ALLEN JAMES and ANWAR NASIM inherited the problem and studied it in Ottawa. With patience and skill with the micromanipulator of the sort that JAMES displayed, it was possible to show that single cells with replicating instabilities seemed indeed to exist. Some time later, and with some reluctance, for it was a mammoth undertaking, JACQUI ROBERTS, working in my lab, managed to repeat this observation. However, not everyone was convinced by these demonstrations and some people still maintained that they were artifactual, so that the origin of mosaic colonies remained hotly disputed for some years. If we accept that they exist, their nature

has still to be finally determined and, even with the availability of molecular techniques, this could be a daunting task.

LOTTE's other main interest in her later years lay in accounting for mutagen specificity. In 1959 she was invited to head the MRC Mutagenesis Research Unit in Edinburgh and she embarked with characteristic enthusiasm on the use of microorganisms for mutagenic studies. Her first choice was Neurospora and she was schooled in its use by KIM ATWOOD, whom I think she failed to understand as a person but for whom she had tremendous regard and respect. During her stay in his lab at the Biology Division of the Oak Ridge National Laboratory in Tennessee, she also met and worked with GUNNAR KØLMARK, who, with NORMAN GILES at Yale and also with MOGENS WESTERGAARD in Copenhagen, had started on a study of mutagen specificity in reversion experiments. At about this time the experiments of BENZER and FREESE and of CRICK, BRENNER and other members of the Cambridge group had laid the molecular basis for mutation and for the genetic code. Impressed though she was by the elucidation of the chemical nature and function of DNA as the genetic material, LOTTE was unimpressed by the almost inevitable tendency of workers studying chemically induced reversion to explain their observations solely in terms of events taking place at the DNA level. Her earlier training ensured that she thought of mutagenesis as a biological process that entailed a series of events of which the nucleotide change, even though essential, was only one. She saw observed mutagen specificities as the product of genetic *and* cellular events, all of which were subject to interference by the mutagenic treatment. These cellular events were thought of as operating at virtually every conceivable level between the exposure of a cell to the mutagen, to the point at which the genetic change occurred, and afterwards in terms of the expression of the altered gene. Successful expression is of particular importance for the treated auxotrophic or sensitive cell, which had both to mutate and to acquire a prototroph or resistance phenotype that would allow it to survive the plating challenge confronting it. Her students and postdocs were all set the task of finding and examining examples of the role of cellular factors in mutagenesis in whatever organism they were using. Out of this work came demonstrations of, for example, the role of constituents in the plating medium in suppressing mutational yield specifically, the inactivation or saturation of repair processes by mutagens that acted as sensitizing agents to their own action, repair specificity at the allelic level, and demonstrations that some less well-defined and probably complex cellular processes may play a role in determining mutational yield. Wherever the search was conducted, it seemed possible to find examples of mutagen specificity attributable to events at the cellular level. LOTTE's greatest moment of triumph, however, came when an undergraduate student, HUGH PATTERSON, was able to show that the inositol mutant allele *inos37401* of Neurospora *could* respond positively when induced to revert with diepoxybutane (DEB). KØLMARK, GILES and others had noted that although this allele reverted readily with UV, it reverted poorly if at all with DEB and other alkylating agents. Many of us privately thought that this was a consequence of an unreactive base pair at the site of the mutation, but at that time we had no way of demonstrating whether this was correct or not. LOTTE, in contrast, always believed that if she could find the right physiological conditions the inositol allele would revert or, rather, its revertants would be expressed. So it was that PATTERSON was given the job of replacing the normal cytoplasmic environment of the strain with the cytoplasm of the *Poky* mutant, a strain with a respiratory defect which is cytoplasmic in nature and maternally inherited. When this was done, and to LOTTE's great delight, inositol reversions were recovered after DEB treatment. When the normal cytoplasm was restored the allele lost its ability to respond again.

LOTTE was always very modest about her scientific contributions, especially about her discovery of the mutagenic effects of mustard gas. As I have said elsewhere, it was clear that she regarded them more as a job of work which she had enjoyed doing. It is perhaps not too great an exaggeration to draw a parallel with one of her favorite composers, J. S. BACH, who regarded himself as a competent craftsman rather than a great composer. She was also as aware as anyone of the limitations of the work she could do. One of my own frustrations was that investigation of the many documented phenomena could not be carried further, and I think it is salutary to note that many of the objectives of those initial studies of chemical mutagenesis were actually achieved in other ways. The work was undertaken originally because it was hoped that the identification of the chemicals that caused mutations would provide sorely needed information on the chemical nature of the genetic partner in the reaction. Once the barrier of demonstrating chemical mutagenesis had been clearly overcome by LOTTE's initial experiments, it soon became obvious that not few but many chemicals of a variety of classes were able to produce mutations. Chemical mutagenesis was simply too blunt a tool for determining the chemical nature of the gene and this was clearly recognized by LOTTE herself. The elucidation of the chemical nature of the gene was achieved by totally different and much more direct approaches. The plethora of mutagenic agents in fact prompted ALEXANDER HOLLAENDER to raise the question of the mutagenic risks from chemicals in the environment, and this spawned the field of genetic toxicology, which for some time LOTTE threw herself into with some enthusiasm. Mutations also played a central part in the elucidation of the genetic code, but this was achieved on the tacit assumption that the specificity of action of base ana-

logues and proflavin owed nothing to cellular processes but simply reflected events at the DNA level. It is true that recognition of the importance of cellular factors in determining mutation yields, repair activity, gene expression, and the metabolism of promutagens, for instance, is no longer a novel idea but part of the intellectual equipment of all those working in the field of mutagenesis, but this realization probably came as much from direct demonstrations as from experiments with Neurospora. Even with these qualifications, however, it is undeniable that the rapid expansion of the study of gene mutation and of chemical mutagens was the result of LOTTE's initial unambiguous demonstration that mustard gas could alter the genes of the fruit fly. Although she hated to be described as the "mother of chemical mutagenesis," in a very real sense she was and this was recognized by the conferring of honorary degrees from the Universities of Leiden, Dublin, Indiana (where MULLER worked), and Cambridge, as well as foreign memberships in the National Academies of Science of the United States and of Denmark.

LOTTE was a passionate believer in the importance of understanding genetics. The subject fascinated her and she conveyed that fascination in her lectures and in her books. She wrote several popular introductions to genetics, some of which were translated into a wide variety of other languages (not all of which she could read, which caused her some concern). Although she had had problems with discipline in her classes as a teacher in Germany, she had no problems as a communicator of scientific ideas. Often with little more than a short list of one-word topics to guide her, she held the undivided attention of successive classes of undergraduates year after year. She spoke with authority but she never minded being questioned. Unlike so many of us these days, she always gave the impression of having all the time needed for discussion. She was not always in control, however, and she used to recall with some amusement the lecture in which the mice being used to demonstrate the genetics of coat color escaped, disconcerting audience and lecturer alike.

She stopped coming to her office in the Institute of Animal Genetics only after she became unable to see to read, and even then she was anxious to learn what was happening in the lab, almost envious of the new opportunities offered by molecular biology and eager to hear what her erstwhile collaborators were now working on. In her last days she had to content herself with listening to music and literature from her tape recorder. She still managed to communicate with her friends and family abroad by phone and letter, but eventually even that became too much. Her death came quickly and without pain. She will be remembered for her love of science, her humility, and her transparent honesty, but by those who knew her well she will be remembered as a faithful and good friend.

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