

Perspectives

Anecdotal, Historical and Critical Commentaries on Genetics

Edited by James F. Crow and William F. Dove

The Discovery of Mustard Gas Mutagenesis by AUERBACH and ROBSON in 1941

Geoffrey Beale

Institute of Cell, Animal and Population Biology, West Mains Road, Edinburgh, EH9 3JN, Scotland

GENETICS in 1940: The notion of the gene as a unit of heredity was well established by 1940, but its material basis was far from clear at that time. Proof of the chromosome theory made it reasonable to believe that genes were components of the chromosomes, presumably proteins, nucleic acids or nucleoproteins. The work of GRIFFITH and AVERY *et al.* showing the special importance of nucleic acids had not yet been assimilated into genetic theory, and the naive concept of chromosomes as "strings of beads" was the best that geneticists of those times could suggest. One of the properties of genes was that of mutation, the change of one form (allele) into another, and it had been shown by MULLER and STADLER that such gene mutation, in addition to occurring spontaneously, could be induced by ionizing radiation (*e.g.*, X-rays) or ultraviolet light. It was thought that such mutation experiments might throw light on the nature of the gene, and in this connection the target theory of TIMOFEEFF-RESSOVSKY, ZIMMER and DELBRÜCK (1935) had been proposed. It was thought possible that the size of the gene might be determined on the basis of this theory. X-ray-induced mutation, however, was found to be mainly the result of inactivation or deletion of genes. The hope had been expressed by MULLER (see below) that some kind of directional mutagenesis might be obtained in the future, perhaps by treating germ cells with chemical substances, and if successful would yield more definite information about the nature of the gene. However, in spite of many attempts, only negative or at best marginally significant results had been obtained from experiments on chemical mutagenesis prior to 1940. The success of AUERBACH and ROBSON in 1941, described in this paper, in obtaining mutations in *Drosophila* by treatment with mustard gas, was therefore an outstanding event in the history of genetics, even though

publication had to be delayed till 1946, after World War II had ended.

The aim of this article is to describe—as far as is possible 50 years after the event—how the discovery was made and to analyze the circumstances leading up to it. As will be shown, these circumstances involved the concurrence of a number of unusual factors, such as political events, war and the chance proximity of key individuals—factors which are not often thought to be responsible for scientific discoveries. It is therefore an excellent example of the unpredictability of some major scientific advances.

AUERBACH: CHARLOTTE (LOTTE) AUERBACH was born in 1899 to a Jewish family in Krefeld on the Rhine in Germany. She went to school in Berlin and, following the custom in Germany at that time, attended lectures in a number of different universities—in Berlin, Würzburg and Freiburg. She remembers receiving instruction from KNIEP in Würzburg and Berlin (in botany), from SPEMANN in Freiburg (Entwicklungsmechanik), and from HEIDER and MAX HARTMANN in Berlin. She took her "Staatsexamen" in 1924 in Berlin and then spent a short time working in developmental physiology under O. MANGOLD at the Kaiser-Wilhelm-Institut in Berlin-Dahlem. However, she found this uncongenial, MANGOLD being a Nazi. On one occasion when she suggested to him that her project might be changed, MANGOLD reacted, according to LOTTE, in a "typical German-Nazi way," saying "Sie sind meine Doktorantin; Sie müssen machen was *ich* sage. Was Sie denken hat nichts damit zu tun." When recounting this incident to me, LOTTE suddenly burst into German, with some feeling. Even after 65 years, MANGOLD's words remained in her mind. As a Jewish woman without private means, she felt she had no chance of making a career in a German university at that time, though with the aid of a small

legacy she had been able to start with MANGOLD.

The only geneticist in Berlin that she remembers is CURT STERN, from whom she received a few lectures, but apparently soon abandoned them to go off singing in a choir. When H. J. MULLER came to Berlin in 1932 and delivered a lecture on the *CIB* technique, LOTTE knew nothing about it.

Her main interest at that time was school-teaching, and after passing the qualifying examination, she spent some years teaching in various schools in Berlin. In 1933, however, HITLER became Chancellor and a law was passed prohibiting the employment of Jews in all state schools in Germany. LOTTE learned about this through the newspapers, and was not allowed to return to her school to collect her belongings. After that she had the opportunity of teaching in a school for Jews only, but was advised against this by her mother—fortunately for, as she later learned, those who did this were afterward killed by the Nazis.

In view of the threatening situation in Germany, especially for Jews, LOTTE decided to leave the country. Through a close Anglo-German family friend (H. FREUNDLICH, Professor of Chemistry in London) she arranged to come to Britain in 1933. Initially she wanted to go to Cambridge and study embryology under C. H. WADDINGTON, but this was not possible as it could have involved too many years of study for a Ph.D. degree. Through FREUNDLICH and G. BARGER (Professor of Chemistry in Relation to Medicine at Edinburgh), she was introduced to F. A. E. CREW, head of the Institute of Animal Genetics at Edinburgh. This Institute was the subject of an earlier essay (FALCONER 1993). CREW offered LOTTE a very modest position at his Institute. On arrival, he gave her some reprints of papers on *Drosophila* and casually invited her to choose a project. She decided to study the development of the legs of *Drosophila*. After two years, in 1935, she wrote a thesis entitled "Development of the legs, wings and halteres in wild type and certain mutant strains of *D. melanogaster*" and was awarded a Ph.D. by the University of Edinburgh.

After this, CREW tried to persuade LOTTE to move elsewhere but, thanks again to the intercession of BARGER, was persuaded to keep her on to look after the mice and especially the budgerigars in which CREW was particularly interested (see FALCONER 1993). LOTTE was also able to get a little more money by acting as an assistant to H. P. DONALD, working on pig records, and giving evening classes in biology.

It happened that the Institute was one of the few places in Britain at that time where genetics research was going on. Plant genetics was being actively pursued at the John Innes Horticultural Institution at Merton, S. London. At Cambridge, it was alleged by the irreverent that the Professor of Genetics, R. C. PUNNETT, was more interested in social activities and

tennis than genetics. But in Edinburgh, CREW had collected a group of what LOTTE called "waifs and strays" on minimal salaries, from various continental European countries—notably P. C. KOLLER from Hungary, G. PONTECORVO from Italy, R. LAMY from Trinidad, and later B. M. SLYZINSKI and H. SLYZINSKA from Poland. Most important of all was H. J. MULLER who arrived in Edinburgh from the USSR and Spain in 1938. The intellectual atmosphere was very lively, and CREW liked everyone to stay in the laboratory late at night discussing their work (and other things), drinking tea or coffee, and even playing ping pong. With the help of her colleagues, especially LAMY and KOLLER, LOTTE taught herself some genetics and did some experiments with *Drosophila* and mice.

At the outbreak of World War II in 1939, with CREW's help LOTTE was able to acquire British nationality and was therefore spared the fate of many distinguished German and Italian scientists of being incarcerated in an internment camp on the Isle of Man. However, on one occasion she did receive a visit from the police, who had been informed that mysterious tapping noises had been heard late at night coming from a room occupied by a lady with a strong German accent. At that time—especially after the fall of France to HITLER's troops—there was a paranoid spy fever in Britain, and it was expected that German parachutists (possibly disguised as nuns) would be descending from the skies. Fortunately, LOTTE was able to persuade the police that her tapping was quite innocent and came from her typewriter. The professor of astronomy, a personal friend, was able to certify that she was not an enemy agent.

Some time in 1938, CREW brought MULLER to LOTTE's room and peremptorily announced that she was to work with him. Later, however, MULLER returned and said it was up to LOTTE to do what she thought interesting. He sat down and discussed her work. He thought that future developments in genetics, particularly with regard to the nature of the gene, would be more likely to follow from studies on mutation than from the developmental studies which had previously been LOTTE's main interest (see AUERBACH 1978). MULLER advised her to try to obtain mutations in *Drosophila* by chemical treatments, and suggested that a number of carcinogens should be tested. LOTTE carried out such experiments with three known carcinogens, 1:2:5:6-dibenzanthracene, 9:10-dimethyl-1:2-benzanthracene, and methyl-cholanthrene, but obtained only negative results (AUERBACH 1940). Following these attempts, LOTTE began experiments with mustard gas, as will be discussed in the next section.

LOTTE received the prestigious D.Sc. from the University of Edinburgh in 1947, largely for her published work on chemical mutagenesis, and continued

research in this area for the remainder of her career, publishing many papers and several books. She was elected a Fellow of the Royal Society of Edinburgh in 1949 and of London in 1957, and in 1970 she became a Foreign Associate of the U.S. National Academy of Sciences. After acquiring a worldwide reputation, she received invitations to return to Germany to take up a senior position there. She declined these offers, however, and informed me that she would "rather work as a lab girl in Scotland than as a professor in Germany."

In 1969 LOTTE became an Emeritus Professor of the University of Edinburgh, but continued to supervise research on mutagenesis even then. Now aged 93, she still lives in Edinburgh and maintains a lively interest in science though she is much handicapped by poor eyesight. She is much loved and respected by us all.

ROBSON: J. M. ROBSON (see ADAM 1984) (hereafter denoted "RAB") was born in Belgium in 1900 to a Russian family named RABINOVICH. He came to England before World War I and attended school and university in Leeds, where he studied medicine. In 1929 he moved to Edinburgh, changing his name to ROBSON on the advice of CREW, and was appointed an assistant to B. P. WIESNER who was working on gonadotrophic hormones at the Institute of Animal Genetics. For a time RAB was in charge of a unit on pregnancy diagnosis, which CREW had set up. In 1934, however, the grant for work on sex hormones was withdrawn and RAB then moved to the Pharmacology Department in Edinburgh (headed by A. J. CLARK). RAB was a man with many interests, and worked on toxicology and chemotherapy as well as sex hormones. Some of his work led ultimately to the development of the contraceptive pill.

In one experiment, which was apparently never published (see ADAM 1984), RAB applied hormonal stimulation to the vaginal epithelium of rats, some of which were exposed to minute doses of mustard gas. The hormonal treatment alone produced a burst of mitoses, but this was inhibited by mustard gas. Thus, RAB showed that mustard gas, like X-rays, inhibited mitosis. The question was whether mustard gas did this by causing lesions in the chromosomes.

In 1940 RAB began a collaboration with LOTTE on the mutagenic effect of mustard gas, as will be described in detail below. In 1946 he moved to the Pharmacology Department at Guy's Hospital Medical School in London, and continued to work on sex hormones and other things, but did nothing further in genetics. He died in 1982.

The experiments of AUERBACH and ROBSON on mutagenesis with mustard gas: The first experiments on the effect of mustard gas on *Drosophila* were done under conditions that now seem extraordinarily prim-

itive. The initial exposures were done on the roof of the Pharmacology Department in Edinburgh. Liquid mustard gas was heated over a bunsen burner in an open vessel, and the flies were exposed to the gas in a large chamber. As a result, all of the people doing this work developed serious burns on their hands, which were then treated with gentian violet. After a short time LOTTE was warned by a dermatologist not to expose her hands to mustard gas any more or she would develop serious injuries. RAB took to wearing gloves. On one occasion LOTTE recalls how she went into a room where RAB and his wife SARAH were eating lunch and said, "There must be mustard gas here. I can smell it." "Nonsense," was RAB's answer. Then LOTTE went round the room and found a beaker of mustard gas bubbling away over a gas burner, filling the room with what she described as a "garlic-like" smell. This did not seem to worry RAB at all. Later, LOTTE left all the exposure work to RAB or his assistants. One of the latter, M. GINSBURG, who was then a Ph.D. student (later becoming Professor of Pharmacology at King's College, London) told me that he was "ordered" by RAB to expose flies to mustard gas.

Eventually a somewhat safer system was devised (AUERBACH and ROBSON 1947a). Nevertheless, it was never possible to control precisely the amount of mustard gas to which the flies were exposed. Sometimes all were killed. After treatment, the survivors, if any, were taken by LOTTE from the Pharmacology Department to the Institute of Animal Genetics, about two miles away in another part of the University, and analyzed by MULLER's *CIB* method, which detects X-linked visible and lethal mutations.

The experiments were begun in November, 1940. On February 1, 1941, LOTTE wrote to MULLER, who had moved to Amherst College from Edinburgh in 1940 and was aware of the work planned by LOTTE and RAB. It was therefore unnecessary to use the words "mustard gas" in letters (which would have contravened the secrecy rule imposed by government regulation).

Dear Dr. Muller and Thea,

In November I started the experiments suggested by CLARK. Unfortunately I had not been warned sufficiently of the danger . . . I was punished with an allergic rash on both hands . . . which lasted many weeks. The substance appears to have many similarities to X-rays . . . It seems worth while trying its possible effect on mutation rates. ROBSON has promised to do the exposures for me . . . and if I treat mature sperm and use the *CIB* method I think I may manage to keep the labour involved within the limits at my disposal. Ponte [G. PONTECORVO, who was no longer in Edinburgh; he was interned from June, 1940 till January, 1941] would have been a person with whom one can discuss *Drosophila* work and plans. There is nobody else in the Institute with whom this can be done satisfactorily. Peo [P.

C. KOLLER] is, of course, absolutely unfitted for his role of director of *Drosophila* research

Yours, as always,
Lotte A.

In April, 1941 the first tests were carried out, as mentioned in another letter to MULLER dated June 7, 1941:

Dear Dr. Muller,

ROBSON was right after all with his hunch about his substance . . . I got heaps of lethals and am quite excited . . . Doses which are not lethal for imagines prevent eggs exposed in the body of the mother from hatching and sperm exposed in the body of the father from fertilizing eggs . . . but the sperm is not killed as the spermathecae of [untreated] ♀♀ mated to treated ♂♂ contain plenty of motile sperm . . . The observations on ♂♂ and ♀♀ taken together suggest that treatment interferes with the orderly process of cell division. This gives one courage to undertake a large-scale mutation experiment using the *CIB* method and a dose which does not interfere too much with fertility of ♂♂ (reduced to something like 1/2 of normal). The following mutation rate was obtained (the figures are not quite final yet).

	<u>Nbr. of chromo-</u> <u>somes tested</u>	<u>Nbr. of</u> <u>lethals</u>
Controls	1216	3
Treated	1213	93

In addition there were 10 semilethals among the offspring from treated fathers, none among the controls, most having visible abnormalities.

Robson has been doing all the exposures for me, as I have become hypersensitive. He is very triumphant that his idea came right. They [*i.e.*, RAB and SARAH] often talk of you and Thea [Mrs. MULLER]

With kind regards, as always,
Lotte Auerbach

MULLER responded by sending a congratulatory telegram in June, 1941. This cannot be traced now but LOTTE thinks it stated, "Congratulations on your major discovery," and says this telegram was "her greatest reward" as MULLER was her hero.

After this first result, LOTTE had a lot of trouble repeating the experiment. RAB insisted this should be done before sending an official report to the Ministry of Supply. On March 27, 1942, LOTTE again wrote to MULLER:

Dear Dr. Muller,

I am afraid you will be disappointed how little further I have progressed with my work since I last wrote. It was not however my fault through laziness, but terrible difficulties with the dosage. From May to December I have done one experiment after the other without being able to reproduce the right dosage again . . . Robson insisted on a repetition of the original experiment for sex-linked lethals. As I expected, the result when it at last came off was completely confirmatory: 68 lethals and four semi-lethals in 790 tested

chromosomes. In addition there were some visible mutations among the semi-lethals

Yours very sincerely,
Lotte Auerbach

Apparently MULLER did not reply to LOTTE's letter of March 27, 1942 (or perhaps his reply was sunk crossing the Atlantic), for on January 29, 1943 LOTTE wrote again:

Dear Dr. Muller,

. . . I hope you won't think me ungrateful if I admit that in spite of my pleasure I was disappointed that there was no word from you or Thea . . . It is such a long time since we last heard from you, and I often wonder how life is treating you two just now. I hope, very kindly. In any case, here are my sincerest wishes that it will do so in 1943.

I also was hoping for a word from you on my work. I am getting rather discouraged by the lack of interest I encounter everywhere. And the fact that you don't write about it makes me suspect that I have disappointed you very much by my various reports . . .

All the same—heartily thanks once more, and the kindest regards for Thea and you.

Yours,
Lotte A.

Of course, it was unreasonable to expect much interest in the work from others since nothing could be published, and even when talking in the laboratory the words "mustard gas" could not be mentioned. Instead, the expression "substance H" was used.

On March 14, 1942, the results of the first experiment were sent to the Ministry of Supply in London (headed by LORD BEAVERBROOK). In the first report, LOTTE and RAB stated, as mentioned above, that sex-linked lethal mutations had been produced by treatment with substance H, as well as breaks and rearrangements of chromosomes as shown by the occurrence of inversions and translocations. The ratio of translocations to sex-linked lethals was considerably smaller than would have been obtained by equivalent doses of X-rays.

In the second report (June 4, 1942), some differences in susceptibility to mustard gas of different *Drosophila* strains were mentioned. It was shown that the basis of these differences was not the chromosomal constitution of the sperm, but genetically determined differences presumed to act "by way of anatomy, metabolism, and behavior." In the third report, sent the same day, it was shown that mustard gas acted directly on the chromosomes rather than by some indirect effect on treated cytoplasm. In the fourth report, again sent on June 4, 1942, the induction of visible mutations was described. This report also contained a preliminary general discussion of the genetic action of mustard gas, thus: "The main genetical effects produced by X-rays, namely lethals, visible

mutations, gross and minute chromosomal re-arrangements, are obtained after mustard gas treatment. So far only three differences from X-ray effects have been observed, namely: (1) a relative shortage of translocations, (2) a high percentage of fractionals, and (3) the absence of certain visible mutations, which however is based on too small a sample to be taken as more than suggestive."

A fifth report (December 23, 1942) dealt with the action of certain other vesicant substances. Negative results were obtained with Lewisite and osmic acid, while ammonia had a slight effect which was not, however, proved to be significant.

Nothing seems to be known about the reaction of the Ministry of Supply to these reports. Presumably their significance for the war effort was considered to be negligible.

Publication: After the war, permission was at last given to LOTTE and RAB to publish their work. Even before this, however, a hint was dropped in a letter to *Nature* (AUERBACH and ROBSON 1944) where it was mentioned that "in the course of the last few years we have examined a number of chemical substances for their ability to produce mutations. Some substances were found to be highly effective, producing mutation rates of the same order as those with X-rays, 6–24 percent sex-linked lethals developing in treated X-chromosomes. These data will be published later." They went on to report that they had obtained mutations by treatment with a substance, allyl isothiocyanate, a naturally occurring mustard oil found in Brassica and other plants. The inference from this was that some "spontaneous" mutations might be caused by chemical mutagens occurring naturally in plants and also that mustard gas itself was mutagenic.

On January 4, 1944, LOTTE attended a meeting of the (British) Genetical Society in London, and presented a paper entitled "The effects of chemicals on the chromosomes of *Drosophila*." How much was revealed at that meeting is not known, but she may have mentioned mustard gas, because J. B. S. HALDANE complained that she should not have done so in public.

When permission to publish was given in 1946, LOTTE and RAB sent another letter to *Nature* (AUERBACH and ROBSON 1946) stating, "in a previous letter, chemical substances were mentioned which were as effective as X-rays in inducing mutations and chromosome re-arrangements. The chemical nature of the main substance used can now be stated. It is dichlorodiethyl-sulphide or mustard gas." This was followed by more detailed accounts in the *Proceedings of the Royal Society of Edinburgh* (AUERBACH and ROBSON 1947a,b). LOTTE also wrote a paper by herself (AUERBACH 1947), which she now considers the most important one, dealing with the induction of chromosomal

instabilities in *Drosophila*. Finally, at the 8th International Congress of Genetics in Stockholm in 1948, LOTTE delivered a comprehensive review paper entitled "Chemical induction of mutations" (AUERBACH 1949). At the same meeting the President, MULLER, stated: "We shall perhaps mention first the dramatic opening up by Auerbach and Robson of the great field of chemical mutagenesis." This gave LOTTE enormous satisfaction.

Priorities in formulating proposals for work on chemical mutagenesis with mustard gas: It is interesting to consider, in retrospect, how it came about that so unlikely and hazardous a substance as mustard gas came to be used as a chemical mutagen. According to LOTTE's statement to me, the originator of the idea was the pharmacologist A. J. CLARK, who had earlier been interested in the biological effects of radiation. At the outbreak of World War II he had a research contract with the Chemical Defense Establishment of the British War Office to study the effects of mustard gas, especially in regard to eye injuries. At that time it was generally expected that gas warfare would be resorted to in the coming war, as it had been in the war of 1914–18. Those of us who were around in 1939, at the beginning of World War II, remember how we were compelled to carry around gas masks in square cardboard boxes, wherever we went at that time. Actually, gas was never used in that war and the boxes were later used for other, more innocent purposes.

According to LOTTE, CLARK was impressed by the long-lasting effects of mustard gas. It produced wounds that were slow to heal and liable to break out again later. In 1939, ophthalmologists were still treating ulcers of the cornea that had been produced by mustard gas in World War I. These long-lasting effects seemed to resemble the somatic effects of X-rays. Thus CLARK got the idea that mustard gas, like X-rays, whose mutagenic action had been shown by MULLER and others, might act on the genetic material in the cell nuclei.

Sometime in 1940, CLARK summoned LOTTE and P. C. KOLLER from the Institute of Animal Genetics to the Pharmacology Department to discuss the possibility of analyzing the effect of mustard gas by genetic tests. According to LOTTE, RAB was not present at this first meeting. Following this and later discussions among CLARK, LOTTE, RAB and KOLLER, it was decided that genetic tests on *Drosophila* exposed to mustard gas should be done by LOTTE in collaboration with RAB, while KOLLER should carry out cytological studies on the chromosomes of *Tradescantia* pollen in flowers treated with mustard gas. KOLLER was initially rather half-hearted about the work on *Tradescantia* when he announced, with his very characteristic Hungarian accent, "All the chromosomes are broken."

Later, however, this part of the work was done at the John Innes Horticultural Institution, then at Merton, South London, in collaboration with C. D. DARLINGTON (DARLINGTON and KOLLER 1947).

The situation in Edinburgh was uniquely favorable for this work. CLARK, who died in 1942, had supplies of mustard gas, and the Institute of Animal Genetics was one of the few places in the U.K. where research in genetics was going on. The presence of MULLER, even for a short time, was outstandingly important. He, however, was unwilling to embark on work with chemical mutagens at that time. Although (as previously mentioned) LOTTE's position was very poor and she had to spend most of her time looking after mice and other experimental animals, her experiments with RAB were successful in spite of many difficulties.

In the genetics literature, the credit for the work on the mutagenic effect of mustard gas is mostly given to LOTTE. A recent Russian visitor to Edinburgh dramatically exclaimed to LOTTE, "You are the mother of mutagenesis" (the "father" being the Soviet scientist I. A. RAPOPORT). For her work, LOTTE was awarded the Keith Prize of the Royal Society of Edinburgh in 1948. In making the award, the President, SIR WILLIAM WRIGHT SMITH, stated:

Miss Auerbach has contributed extensively to our knowledge of mutation processes. Many of the results of her investigations have been published in the Proceedings of the Society . . . she is distinguished for her researches into the genetical effect of the mustard gases, and in these experiments was associated closely with Dr. Robson . . . Her publications in the Society have attracted world-wide notice. It might be of some interest to state that Dr. Auerbach received her training for this special field of research from Professor Muller, Nobel Laureate . . .

This award caused great offense to RAB and even more to his wife SARAH, who wrote a furious letter to LOTTE complaining that the prize should have been awarded jointly to LOTTE and RAB. LOTTE informed me that she wrote back apologetically, "I completely agree with you, but I had to accept the prize because I needed the money" (actually only £50). LOTTE later stated that "They never forgave me," and she did not succeed in having any further contact with the ROBSONS, who had left for London in 1946. RAB's pique is fully understandable. In an interview in 1971, he stated (rather bombastically, perhaps),

I discovered the mutagenic action of mustard gas. I had the idea that mustard gas would be mutagenic. I suggested to Muller he should do it. He suggested I should approach Lotte Auerbach . . . I knew Joe Muller very well. My wife and I were the only people at the wedding [of MULLER and THEA] . . . (Taped unpublished interview with MARGARET DEACON in 1974, quoted by permission.)

LOTTE herself on numerous occasions has stated that she does not wish to be remembered principally

for the discovery of mustard gas mutagenesis, modestly pointing out that, had she not done it, someone else would have, and in any case this was only one chemical among many others which were shown to be mutagenic then or later. She prefers to be remembered for her subsequent work on the study of mutagenesis in depth, especially on the production of "replicating instabilities."

No doubt many factors contributed to the proposal (and successful prosecution) of the work. Among these we may mention: (1) the imminence of World War II, leading to the support for CLARK's work on mustard gas in Edinburgh; (2) the presence in Edinburgh at the same time of CLARK, RAB, LOTTE, and above all MULLER, who met frequently for discussion of research; and (3) the existence of CREW's Institute of Animal Genetics, at which there was a lively group creating an atmosphere favorable for scientific work. LOTTE herself informed me that, speaking ironically, even HITLER could be held to some degree responsible, as he had forced her to leave Germany and abandon school teaching for scientific research. But there seems no doubt that it was the expertise in *Drosophila* research methods and perseverance of LOTTE along with the intrepid, perhaps foolhardy handling of the chemical that were mainly responsible for the success of the work.

Discussion: After World War II was over and communications were reestablished between scientists in different European countries, it became apparent that several other chemical substances, in addition to mustard gas, had been shown to be capable of breaking chromosomes and/or causing gene mutations. Among these substances, the most significant was probably urethane, which was studied extensively by OEHLKERS (1943, 1949) in Germany and found to cause chromosome breaks in *Oenothera*, and was also found by VOGT (1948) to cause recessive lethal and visible mutations in *Drosophila*. According to LOTTE, OEHLKERS felt very hurt that she was always given the credit for the discovery of chemical mutagenesis, and not he.

Formaldehyde (in the food) was shown by RAPOPORT (1946) in the USSR to produce mutations in *Drosophila* larvae, though this work was much hindered by the rise to power of T. D. LYSENKO, who attained total domination over biology in the USSR during and for some years after World War II. RAPOPORT was strongly opposed to LYSENKO's crazy ideas and consequently was prevented from carrying on research on mutagenesis. Another mutagenic substance was phenol, discovered in Switzerland during the war by HADORN (1949) to produce mutations in *Drosophila* ovaries treated in the third instar larval stage. These results, however, were uncontrollably variable; numerous visible mutations were produced in some experiments, but none were found following

treatment of males subsequently tested by the *CIB* method. LOTTE says that HADORN was at first suspicious that she wanted to "hog" the field of mutagenesis.

Subsequently, of course, many other substances have been shown to be mutagenic (see AUERBACH 1976). It is beyond the scope of this article to discuss them. Expansion of the work on mutagenesis was particularly great after the discovery of the structure of DNA by WATSON and CRICK in 1953, which made possible a more rational approach than previously. In this connection, however, LOTTE expressed herself trenchantly. She wrote (AUERBACH 1978),

... mutation is a process that takes place inside living cells, and as such cannot be described fully by a purely physical or chemical model, however ingenious, and the advent of the double helix has meant a tremendous break-through in our understanding of mutation, but it has neither been the beginning nor the end of mutation research. One gets the impression that molecular geneticists labour under two misapprehensions: (1) that no meaningful questions about mutation could be asked, even less answered, before 1953, and (2) that all questions were answered by the Watson-Crick model....

Probably one reason for this outburst was the failure of LOTTE or anyone else to explain some aspects of chemical mutagenesis in terms of base substitutions or other changes in DNA structure. This applies particularly to the tendency of mustard gas and other chemicals to produce the so-called "replicating instabilities," that is, unstable genes that not only continue over many cell cycles to throw off the same mutation, but also can replicate the unstable state. This phenomenon continued to be studied by LOTTE and her research group at Edinburgh right up to the time of her retirement, but was never satisfactorily explained. Thus, it is to some extent understandable that she was hostile to the idea that mutagenesis could be interpreted solely in physical or chemical terms, though such an attitude in this matter is quite unfashionable at the present time. The mechanism of replicating instabilities remains unknown.

In conclusion, it should be pointed out that the main significance of the work of AUERBACH and ROBSON is the clear demonstration that gene mutations can be induced by treatment of *Drosophila* with a chemical agent, mustard gas. Although the discovery did not increase our knowledge of the chemical nature of the gene, as had earlier been thought possible, it led to the later discovery of many other chemical mutagens which became vitally important tools for genetic research. It also had an important impact on the use of some chemicals, such as nitrogen mustard and other alkylating agents, in the treatment of cancer. Most remarkable, perhaps, were the unusual cir-

cumstances leading to the planning of the work and the long period during which the results remained classified as "secret."

The author acknowledges the enormous help he has received from CHARLOTTE AUERBACH in preparing this paper and allowing him to quote excerpts from her letters to H. J. MULLER. Grateful thanks are also extended to HENRY ADAM (Edinburgh) and MICHAEL GINSBURG (London) for their recollections of ROBSON, and to MARGARET DEACON (Mrs. SEWARD) and the Science Studies Unit of Edinburgh University, for permission to use some information in her unpublished report and taped interviews on the history of the Institute of Animal Genetics, Edinburgh. The excerpts from correspondence between AUERBACH and MULLER are quoted from the MULLER Mss. by kind permission of the Lilly Library, Indiana University.

LITERATURE CITED

- ADAM, H., 1984 John Michael Robson. Obit. Not. R. Soc. Edinb., pp. 209-212.
- AUERBACH, C., 1940 Tests of carcinogenic substances in relation to the production of mutations in *Drosophila melanogaster*. Proc. R. Soc. Edinb. B 60: 164-173.
- AUERBACH, C., 1947 The induction by mustard gas of chromosomal instabilities in *Drosophila melanogaster*. Proc. R. Soc. Edinb. B 62: 307-320.
- AUERBACH, C., 1949 Chemical induction of mutations. Proc. 8th Intern. Congr. Genet. Hereditas (Suppl. Vol.), pp. 128-147.
- AUERBACH, C., 1976 *Mutation Research*. Chapman & Hall, London.
- AUERBACH, C., 1978 Forty years of mutation research. A pilgrim's progress. Heredity 40: 177-187.
- AUERBACH, C., and J. M. ROBSON, 1944 Production of mutations by allyl isothiocyanate. Nature 154: 81.
- AUERBACH, C., and J. M. ROBSON, 1946 Chemical production of mutations. Nature 157: 302.
- AUERBACH, C., and J. M. ROBSON, 1947a The production of mutations by chemical substances. Proc. R. Soc. Edinb. B 62: 27.
- AUERBACH, C., and J. M. ROBSON, 1947b Tests for chemical substances for mutagenic action. Proc. R. Soc. Edinb. B 62: 284-291.
- DARLINGTON, C. D., and P. C. KOLLER, 1947 The chemical breakage of chromosomes. Heredity 1: 187-221.
- FALCONER, D., 1993 Quantitative genetics in Edinburgh: 1947-1980. Genetics 133: 137-142.
- HADORN, E., G. BERTANI and S. ROSIN, 1949 Ergebnisse der Mutationsversuche mit chemischer Behandlung von *Drosophila* ovarien *in vitro*. Proc. 8th Intern. Congr. Genet. Hereditas (Suppl. Vol.), pp. 256-266.
- OEHLKERS, F., 1943 Die Auslösung von Chromosomenmutationen in der Meiosis durch Einwirkung von Chemikalien. Z. Indukt. Abstammungs. Vererbungslehre 81: 313-341.
- OEHLKERS, F., 1949 Mutationsauslösung durch Chemikalien und die Bedeutung des Plasmas. Proc. 8th Intern. Congr. Genet. Hereditas (Suppl. Vol.), pp. 635-637.
- RAPOPORT, I. A., 1946 Carbonyl compounds and the chemical mechanism of mutations. Dok. Acad. Nauk. SSSR, Ser. Biol. 54: 65-67.
- TIMOFEEFF-RESSOVSKY, N. W., K. G. ZIMMER and M. DELBRÜCK, 1935 Über die Natur der Genmutation und der Genstruktur. Nachr. Ges. Wiss. Göttingen (Math. Physik. Klass. Fachgruppen VI Biol.) 1: 234-241.
- VOGT, M., 1948 Mutationsauslösung bei *Drosophila* durch Äthylurethan. Experientia 4: 68-69.