General Discussion

In the introduction of the thesis a survey of the literatures on the effects of ultraviolet and other radiations and chemical mutagens on various groups of fungi was made. The evidence thus provided together with the facts that the study of genetics of fungi is fundamentally dependent upon the sequence of events almost basically similar to those we come across in higher organisms, strongly suggest that there is ample scope for the study of the mutational behaviour of ascomycetous fungi with or without regular sexual cycle. In the earlier phase of research work on mutation the genetic analysis in a strain of fungi was considered difficult as the techniques for this specific problem were not fully developed until Lindgren and Lindgren (1941) and Beadle and Tatum (1941) successfully laid down the procedures for the study of biochemical mutation in Neurospora crassa. The generalised research methods have been well-adopted in the present case and a few minor modifications were introduced with a view to enhancing the speed of work.

The selection of the strain of fungi was done by the author from a tentative study of the species of Aspergillus available in stock and the final selection was made in favour of Aspergillus Chevalieri which was considered suitable and adaptable for mutational and genetical work.

In the experimental results presented in Chapter I of the thesis it was clearly indicated that the chosen strain of Aspergillus Chevalieri (72070) had two important genetic markers e.g. colour of conidia and production...
of a chemical substance showing antibiotic properties against bacteria and fungi in vitro studies. The growth and metabolism of the strain under investigation was not substantially affected by the change in the environmental conditions and the antibiotic substance remained stable at higher temperature without any marked impairment of its antibiotic activity or production of confidial colour.

The selected strain of *A. chevalieri* was subjected to ultraviolet radiation at the fixed wavelength 2537 Å and the mutants, both morphological and biochemical were raised in quite a good number. The combined effects of UV radiation and treatment with nitrogen mustard were quite interesting and from both experiments, a wide range of deficiency mutants were isolated. There has been no significant difference in the types of mutants obtained from nitrogen mustard and UV irradiation series except the occurrence of 2 strains of histidine deficient mutants (Table 6) of *A. chevalieri* which was worthy of consideration. It will be evident from comparative data (Tables 4 and 6) that nitrogen mustard is not equally efficient as compared to UV rays specially with respect to the strain of *Aspergillus chevalieri* employed here for mutation work. Similar results were obtained by Bogan et al. (1954) on *Penicillium chrysogenum* and Hollroyd et al. (1947) on *Neurospora crassa*. There has been disagreement as well with the results obtained with *Neurospora crassa* and *Penicillium nattus* (Horovitz et al., 1946 and Stahlmann and Stauffer, 1947) with regard to the effectiveness of nitrogen mustard employed as a chemical mutagen.
The study on the mechanism of the action of nitrogen mustard on the spores of *A. chevalieri* could not be undertaken but the widely accepted hypothesis seemed to be applicable. The above chemical mutagen was known to possess definite inhibitory properties against cancer cells and certain types of leukemia and that was one of the reasons for its use as a mutagenic substance by geneticists. The reactivity of nitrogen mustard has been known specially with the sulphhydryl and amino groups of several amino acids including arginine, threonine, methionine and histidine (Kinsey and Grant, 1946 and Hartwell, 1946). The frequency of morphological mutation was significantly higher than the biochemical mutation in most of the experiments. This is probably due to the fact that the phenotypic characters are controlled by several genes.

In a separate set of experiments the modifying effects of UV radiation was studied and it was demonstrated that pre- and post-irradiation treatments with nitrogen mustard caused modification of the mutagenic effect. Pre-treatments caused enhanced mutation frequency in the present case and this is in agreement with the results reported by Swanson and Goodgal (1948) on *A. terreus* and Swanson, McElroy and Miller (1949) on *Neurospora* spp.

The radiation damage to biological material is known to be dependent on various environmental conditions and chemical modifiers. This is true for pre-, during and post-irradiation treatments which either tends to accentuate or decrease the effects of radiations. In *A. chevalieri* it has been possible to demonstrate such effects but the chemicals used as modifying agents are all nutrient substances except nitrogen mustard which is a chemical mutagen.
Our knowledge about the modification of the biological effects of UV rays is not quite sufficient to draw any general conclusion, and the variability of the strains of microorganisms to similar treatments warrants a detailed investigation on the process involved. The experimental evidence presented above on the modification of the damaging effects of UV irradiation with respect to only one strain of fungi, e.g., *Aspergillus chevalieri* (73070) will contribute only to a limited extent to our understanding of the nature of biological effects of UV radiation.

While considering the characteristics of morphological mutants derived from *A. chevalieri* (73070) through UV irradiation and by treatment with nitrogen mustard the author has been able to classify 1227 morphological mutants into seven broad groups with quite a good number of subgroups. The selected strains showed distinct color and colony variations. The stability of the mutants were determined and unstable ones were rejected at the start. These changes could be assigned to be chromosomal mutation but as the crossing experiment with the parent strain of the *Aspergillus chevalieri* could not be done it is difficult to make a conclusion in this respect. However, paro-sexual mechanism has been studied in the present strain and the results are given in another chapter of the thesis. Extensive work on koji molds *Aspergillus oryzae* and *Aspergillus sojae* on morphological mutation was done by Inohama and Sakaguchi (1955) and they had thoroughly evaluated the extent of variation in the molds used by them. The color of the conidia, nature of sporulation and growth rate of the colony were the principal morphological.
changes observed by the investigators. The results obtained here with *A. chevalieri* are in good agreement with those obtained earlier.

The parent strain *A. chevalieri* (78070) had been known to produce an antibiotic in liquid medium and this was assigned as a genetic marker. Consequently the variation in the genetic constitution was expected to induce a change in the character for antibiotic production. Although the production of the antibiotic could be said to be absolutely controlled by genes of the organisms, we must take into account other factors also which would possibly control the synthesis of this metabolic product. However, the cultural and growth conditions of the parent strain and its mutants were maintained without any significant variation in the present case. From Table 15a, it will be evident that the parent strain 78070 could produce antibiotic substances inhibiting all the four test organisms while the antibiotics produced by its four morphological mutants did not show any such activity against *S. aureus* and *B. subtilis*. *Salmonella typhosa* was found to be extremely sensitive to the action of the antibiotic and that is why this was selected as an assay organism in all future experiments.

The phenomenon of the biosynthesis of several antibiotics by one organism could be explained by the hypothesis that one and the same organism must be producing different antibiotics during its growth and metabolism. The experiments on chemical purification and fractionation of antibiotics could not be undertaken in an elaborate manner in the present case and therefore, the plan of research was not extended to the chemical purification of antibiotic but only a preliminary idea of its
chemical nature was worked out. At this stage it would not be possible to furnish sufficient data for establishing the identity of the antibiotics produced by the parent strain of A. chevalieri. More work on the purification of the antibiotic will be necessary for ascertaining the detailed characteristics of the antibiotic. Recently Wilkinson and Spilsbury (1965) reported that a strain of A. chevalieri produced gliotoxin in liquid medium and that was active against bacteria and fungi. The strain of

A. chevalieri employed in the present case produced only antibacterial antibiotics in significant quantity and the antifungal property was almost negligible.

It has been demonstrated that a number of first- and second-step mutants with single or alternate nutritional requirements showed complete loss of capacity to produce antibiotic substances even if the required substances were added in adequate concentration to the culture medium. The auxotrophs showing this specific behaviour were given in Table 29b and the linking of nutritional deficiency was discussed earlier.

The course of biosynthesis of antibiotics by the mutants of Aspergillus chevalieri had been altered because of gene block at different stages of synthesis of cellular constituents. The accumulated precursors in the above mutants have a chance to pass through alternate routes for being utilised by other enzyme mechanisms in the cell system.

It is not difficult to consider however, that the loss of a function in a mutant, i.e., antibiotic production in the present case may be placed
in the same category as an increase in the intensity of the synthetic capacity. The loss of a function involves, in metabolic blocks at particular stages in the synthetic cycle possibly causing the undesired transformation of the intermediate precursor. It is apparent that in the mutant organism imbalance in the functioning of an enzyme will tend to influence other enzymatic systems accompanied by physiological alterations.

In most filamentous fungi the formation of heterokaryons is an evidence of complementation and mutations could be proved to be non-allelic. In the results presented here on the formation of heterokaryon only four pairs of mutants responded to the complementation test and there were many intermediate cases amongst the recombined auxotrophs.

The experiment was performed on a rather restricted scale as there were several difficulties to be surmounted to get consistent results. The formation of a heterozygous diploid was expected but this was recorded only in one instance which later on became unstable and could not be continued. The results obtained in Aspergillus nidulans or Neurospora crassa were not comparable in the present instance as experiments could not be done in the same manner and therefore analysis of perithecia and ascospores had to be abandoned. The principal difficulty was that the perithecial structures are rather small for convenient handling.
Experiments on reverse mutation were done only with one mutant having mutation at the arginine locus and a comparison of the results has been given in Table 35 as to the reversibility of the particular gene with various mutagenic agents. The reason for selecting the arginine mutant for testing their reversibility was that such mutants were obtained in a comparatively higher frequency with both UV radiation and mustard gas treatment. As such it was considered worth while selecting this type of deficient mutants for the study of reversibility of the mutated gene to the wild type character.