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CONTENTS

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BY

K. K. MITRA, B.Sc. (HONS.), A.I.I.Sc., PH.D.

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M. K. SUBRAMANIAM, M.A., D.Sc.

1950

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BY K. K. MITRA, B.SC. (HONS.), A.I.I.SC., PH.D.
AND
M. K. SUBRAMANIAM, M.A., D.SC.
*(Cytogenetics Laboratory, Indian Institute of Science, Bangalore-3)*

CONTENTS



### INTRODUCTION

In a previous contribution (Mitra and Subramaniam, 1949) it was shown that prolonged treatment with chrysene induces tetraploidy in a diploid brewery bottom yeast. The investigation was planned mainly with an idea to evaluate clearly the changes induced by carcinogens. An important angle of approach has been to evolve methods for the genetical improvement of industrially important micro-organisms. The problem of carcinogenesis has naturally to be considered subservient to the general mutagenic property of various chemicals. Parallel investigations in this laboratory on the mutagenic ability of diverse physical and chemical agencies led to the discovery that induction of tetraploidy should be preceded by a gene mutation (Subramaniam and Sreepathi Rao, 1950; Ranganathan and Subramaniam, 1950; Ranganathan, 1950). As such, it is to be expected that agencies inducing polyploidy also accelerate gene mutation.

In the previous contribution the question was posed whether carcinogenic compounds first produce a gene mutation and then polyploidy. This would necessitate a broadening of the definition of mutation. Dobzhansky

(1947) remarks that mutational changes fall into two broad classes. The mutation proper, often spoken of as point mutation, refers to the alteration in an individual gene. The other type involves either physical destruction, spatial rearrangement or duplication of the genes. He has also pointed out: 'An attempt to discriminate between these classes by examination of the chromosomes of the mutant types under the microscope breaks down because of the limitations of the existing cytological techniques. For example, a loss of a chromosome section may be detectable in a species with large and well differentiated chromosomes, but not in one with small and compact ones' (page 21). Tetraploidy itself is considered analogous in its effect to that of a gene mutation since any doubling of the chromosome complement has necessarily to produce a change in the norm of reaction (page 229).

Demerec (1948) evaluated the action of the various chemicals on the basis of two different criteria. He considered a substance to be a mutagen if it produced either a gene mutation or a chromosomal rearrangement. According to Auerbach (1949) this definition of mutagenicity may not be acceptable to every geneticist. Investigations in this laboratory indicate that even the production of a viable translocation should be preceded by the mutation of one or more genes (Prema Bai, 1947; Ranganathan, 1950; Ranganathan and Subramaniam, 1950). It should be obvious that the substance producing chromosome fragmentation need not produce viable chromosomal rearrangements (Levan and Tjio, 1948 *a*, *b*). The viability of a chromosomal translocation necessarily implies that the altered genic sequences are capable of a normal functioning. Thus if gene mutations precede even viable translocations, the mutagenicity of any particular chemical could be judged by either of the criteria suggested by Demerec (1948).

Curiously enough, as would be seen from the observations presented in this paper, chrysene which induces polyploidy also induces visible gene mutations.

Although X-rays and ultra-violet irradiations which induce gene mutations (Muller, 1947) have also been known to produce carcinogenesis (Furth, 1944), the mutagenic ability of chemical carcinogens had not been established for a long time. Strong (1946, 1947) records a variety of hereditary variations in mice treated with subcutaneous injections of methylcholanthrene for a number of generations. In the above experiments he observed the occurrence of simultaneous changes in different directions. He believes that the treatment with methylcholanthrene may have created

an ' unstable ' genetic state. It is known that mice are not as suitable for investigations on mutation rates as *Drosophila* (Beadle, 1948). Demerec (1948) records a significant increase in the mutation rates of *Drosophila* exposed to the action of 1-2-5-6-dibenzanthracene in the form of an aerosol of its solution in oil. The data obtained by him indicated that judged on the basis of the frequency of sex-linked lethals, dibenzanthracene may be considered as a weak mutagen. The surprising results in this series of investigations was the occurrence of chromosomal rearrangements. In a subsequent contribution Demerec (quoted by Auerbach, 1949) seems to have obtained very significant correlation between mutagenic and carcinogenic properties when the definition of mutagenicity was broadened to include chromosomal rearrangement also. These investigations offered some indications regarding the existence of specific chemicals whose activity is limited to the production of chromosomal rearrangements. It may be that some of these chemicals do produce only the specific gene mutations enabling the production of a viable chromosomal translocation. The observations in this laboratory (Ranganathan and Subramaniam, 1950) regarding the mode of origin of the riboflavin excreting top yeast indicates that just as polyploidogens are capable of producing gene mutations as well as chromosomal rearrangements, the effect of certain chemicals may be more specific.

There is an obvious difficulty when a generalisation is attempted on the basis of the effects of the carcinogens on *Drosophila*. " Penetration to the chromosomes and the metabolic fate of the tested substances may well differ so much between mammals on the one hand, and *Drosophila* or micro-organisms on the other, that a positive correlation becomes obscured " (Auerbach, 1949, page 378). It is stated to be quite likely that the carcinogens may act on the specific genes present only in mammals. Investigations on yeast, therefore, can only offer an explanation as to the mode of action of the various chemical mutagens including carcinogens.

Apart from the above difficulties in evaluation, investigators do not seem to have distinguished the effects of the tested mutagens separately on the mitotically dividing cells on the one hand and differentiated tissues which are considered to be endopolyploid, on the other. In the previous contribution it was emphasised that polyploidy and endopolyploidy are not synonymous (Mitra and Subramaniam, 1949). The necessity for a clear evaluation of the effects of any tested chemical on different types of tissues having entirely varying nuclear constitution becomes imperative even in the experiments reported by Auerbach (1949). Developmental stages of

*Drosophila* exposed to a low concentration of mustard gas produced very high mortality. Adult insects alone were able to withstand the general toxic effects. Auerbach suggests that the difference in the reaction of the adult insects to the toxic effect may be due to the preferential action of mutagens on the dividing chromosomes. In an adult *Drosophila* cell division is very rare. White (1945) remarks: "It has frequently been stated that, apart from the special cases like *Ascaris* and *Miastor* the number of chromosomes is the same for all tissues of an organism. In insects at any rate this is certainly not so. Most of the cells of an adult insect have lost the power to divide by mitosis, but their nuclei are highly polyploid, different degrees of polyploidy being characteristic of particular tissues' (page 31). In view of this, the necessity for clearly distinguishing the effects of the various chemicals on differentiated and undifferentiated cells becomes imperative. The conditions in vertebrates may not be different. "In the vertebrates conditions are not favourable for counting the chromosomes in somatic resting nuclei, but measurements of nuclear volumes (Jacobj, 1925, 1935) suggests that in some mammalian tissues several size classes of nuclei exist, and it is possible that these correspond to different degrees of endopolyploidy" (White, 1945, page 34). The suspected divergence in the metabolic fate of the mutagens in *Drosophila* and in mice may simply revolve around the question of the different types of differentiation which have taken place in the two organisms. Whether results on yeast have got a wider application or not, yeasts form very favourable material for the investigation on the effects of mutagens on mitotically dividing as well as endopolyploid cells. Under aerobic conditions yeasts divide mitotically while under conditions of anaerobiosis they become endopolyploid (Subramaniam, 1947, 1948). It is possible, therefore, to test the effect of the chemicals under differing physiological conditions.

The need for such a series of investigations will be apparent from the observations of Caspersson and Santesson (1942). They classify the cells found in malignant growth under different categories. The actively growing 'A' type of cells give rise to the non-growing but differentiating 'B' types. Koller (1947) in his Fig. 2 (page 273) has slightly modified that (Fig. 23) given by Caspersson and Santesson where the 'A' type of cells dividing mitotically give rise to cells undergoing differentiation as a result of endomitosis. It is these endopolyploid cells that later become necrotic and die. If, as suspected, differentiated tissues are endopolyploid, the mitotically dividing cells could not have originated from the differentiated cells. They ought to have taken their origin from the rare, mitotically dividing 'replacement' cells which are found even in differentiated tissues. Only such an

origin could possibly explain the occurrence of mitosis in the cells of the 'A' type and their later transformation into endopolyploid ones. It is surprising to see the remarkable similarity in the composition of the population in differentiated tissues on the one hand and fermenting yeast cultures (Subramaniam, 1947, 1948) on the other. In both the cases only a very microscopic percentage of the cells retain their ability for unlimited proliferation. It has been emphasised by Subramaniam (1947, 1948) that death and disintegration is the fate of the endopolyploid cells, which form the major percentage of population in a fermenting yeast culture. Even in malignant tissues necrosis appears to be the stage succeeding endopolyploidy.

## Material and Methods

The procedure employed for the treatment of the diploid brewery bottom yeast with chrysene was identical with that followed in the previous investigation (Mitra and Subramaniam, 1949). The concentration of the alcoholic solution of the chemical as well as the quantity of the chemical added to the medium were identical. In the previous experiments it was established that the final effect of a prolonged treatment with chrysene is an induction of tetraploidy. In the present series, therefore, interest was mainly confined to an evaluation of the progressive changes in the population on continued exposure to chrysene.

Cells from a liquid culture of the strain grown for 24 hours in barley malt wort formed the starting material for the experiment. One loop from the culture was introduced into a tube containing 5 ml. of wort. Simultaneously with this, another inoculation was made into a similar tube containing 5 ml. of wort incorporated with six drops of the saturated solution of chrysene.

After the incubation period of 16 hours at room temperature the material from the first tube was used for the inoculation of giant colonies according to the method given by Subramaniam and Ranganathan (1948). Such a control colony of the strain at the start of the experiment was necessary in order to compare the changes undergone by the strain as a result of the treatment with the chemical for varying lengths of time. A loop of the material from the second tube, which represented the yeast population after a treatment with the chemical for 24 hours was transferred into 5 ml. wort for the inoculation of the giant colonies on the following day. The rest of the culture in the tube was kept for further treatment. The exposure to the chemical was continued by regular subculture of the strain through medium containing chrysene. Such transfers were made at least every alternate day in order that the maximum percentage of the cells in the population

could be retained in the growing state. At intervals, in the course of the treatment, the probable changes were evaluated by growing giant colonies of the treated samples. The same procedure as described in the case of the culture treated for 24 hours was followed for these inoculations. Samples after specified periods of treatment were tested by growing in ordinary wort for 16 hours. Colonies representing the cross-section of the population at the end of different periods of treatment were thus obtained. All the colonies were grown at the room temperature and to get the correct picture duplicates and often quadruplicates were grown. They were photographed before they turned brown in colour immediately on cessation of growth. The exposure to chrysene was continued till the giant colony of the material showed the characteristic smooth appearance of the tetraploid strains CHR 1 and CHR 2 isolated previously after a similar treatment (Mitra and Subramaniam, 1949).

Since a continuous series of observations by Subramaniam and coworkers (Subramaniam and Ranganathan, 1948: Subramaniam, Ranganathan and Krishna Murthy, 1948) have shown that the giant colony characteristics of the yeast population can be used as a convenient criterion for the location of many of the genetical variations undergone by the control diploid strain, a similar procedure was followed for the evaluation of the results in the present studies also. The identification of the mutations as revealed by the characteristics of the giant colony were based on the studies on reverse mutations in the strain under investigation reported by Subramaniam, *et al.* (1948). To facilitate a proper understanding of the analysis and interpretation of the giant colonies described in the present paper it will be necessary to give an account of the classification of the different types of colonies met with in the case of this strain. Subramaniam, *et al.* (1948) described them as follows:

" *Smooth I* has a wavy or serrated margin with radial folds and with or without very faint concentric striations.

*Smooth II* is similar to the above but has pronounced concentric striations specially in the outer half of the colony.

*Smooth III* has innumerable radial and concentric striations in the outer half of the colony giving it a lace-like texture.

*Rough I* is similar to *Smooth III* with sculpturing limited to the outer half of the colony, but differs from it in having a rough texture owing to the presence of minute powdery granulations on the surface.

*Rough II* resembles *Rough I* in general, but with the difference that the entire surface of the colony has a rough sculpturing" (pages 46–47).

The characteristic smooth nature of the various autotetraploid strains obtained by the treatment of the control with chrysene has already been illustrated in the previous contribution (Mitra and Subramaniam, 1949).

Four independent series of experiments were conducted in the course of about an year and are analysed in the paragraphs that follow.

## Observations

*Experiment I.*—The first series of experiments were conducted during the months of September-October of 1948. The giant colonies were grown in large petri dishes. The observations on reverse mutations recorded by Subramaniam, *et al.* (1948), were on colonies grown in petri dishes of slightly smaller size. The question of genic expression is governed by the quantity of the nutriment available in a given area and hence the thickness of the medium determines the time required for the final expression of the character (Subramaniam, 1950).

The control at the start of the experiment belonged to the *Rough I* type (Photos 1 and 2). After treatment for 24 hours with chrysene the rough sculpturing was not prominent (Photo 3). The duplicates show a condition reminiscent of *Smooth II* (Photo 3) and *Smooth I* (Photo 4). Even such a short treatment seems to have resulted in a change in the population. This picture persists in colonies grown after 5 days of treatment (Photo 5) but as the duplicate (Photo 6) would indicate, the culture had become a mixture of *Smooth II* and *Smooth I*. After 13 days of treatment the *Rough I* appears again in the culture (Photo 7). But that is not all. Photo 8 would indicate the existence in the culture of *Smooth II* and tetraploid cells as well. The predominance of the smoother types during the early part of the experiment does not appear to be the result of any selective action of chrysene. If that was so, the rough type could not have reappeared (Photo 7). That chrysene accelerates mutability could be seen from the *Smooth III* cells found after 13 days of treatment (Photo 9). As would be obvious, the culture after 13 days of treatment is composed of the following types: *Smooth I* and *Smooth II* (Photo 8), *Smooth III* (Photo 9), *Rough I* (Photo 7) and tetraploid (Photo 8).

Since the population was a mixture, quadruplicate colonies (Photos 10, 11, 12 and 13) were inoculated for the material treated for 21 days in order to get a cross-section of the population. Photos 10 and 11 could be assigned to *Smooth I*. Tetraploid sectors are found in Photo 12, but from the shape of the sectors of *Smooth II*. it would appear that they have a much better

growth rate than the tetraploid itself. This is nothing surprising since a gene determining the growth rate in the diploid strain has been described by Subramaniam and Ranganathan (1949). In Photo 13 also the diploid sector shows a better rate of growth.

Such unmodified diploids disappear progressively on continued treatment. Four colonies grown separately after 30 days of treatment are given as Photos 14, 15, 16 and 17. Photos 14, 16 and 17 are typical tetraploids. A comparison of Photo 15 with Photos 10, 11, 12 and 13 would indicate that the tetraploid has a much better rate of growth than the diploid in Photo 15. The diploid persists even after 38 days of treatment and could be seen as sectors in Photos 18 and 19.

*Experiment II.*—It has been the experience of the investigators of chemical mutagens that identical exposures often give entirely different results. If chrysene merely accelerates mutability at different loci even changes in duplicate tubes undergoing treatment may not be identical. This was tested in the second series by a study of the giant colonies from populations in two sets of tubes undergoing identical treatment.

Photos 20 and 21 illustrate the giant colonies of the control at the commencement of this investigation. Both are *Rough II*, only there are two small *Smooth II* sectors in Photo 20. Duplicate colonies from the first set of tubes showed, as in the previous experiment, the appearance of smooth sectors after 5 days of treatment (Photos 22 and 23). In the second series also the picture was the same (Photo 24).

After 12 days of exposure, however, there was a divergence in the nature of the population in the two series. In the first various types like *Rough I, Rough II. Rim* and *Smooth III* could be observed in Photo 25 while *Rough I* and *Smooth II* may be observed in Photo 26. In the case of the second set of tubes *Rough II* cells persist with *Smooth I* sectors (Photo 27). On the 18th day of treatment *Smooth I* cells predominate in the first set (Photo 28) but the *Smooth III* put in their appearance in the second (Photo 29). A comparison of Photos 25 and 29 suggests that there is a delay in the appearance of *Smooth III* in the second set of tubes. After 28 days of treatment the colony of the material from the first series appears to be a mixture of the smoother types with a predominance of cells showing the intricate sculpturing of *Smooth III* (Photo 30). This picture persists even after 34 days (Photo 31) in the first series. In the second set on the other hand, the culture became predominantly tetraploid after 34 days under identical conditions (Photo 32). Photo 33 of a giant colony grown after exposure for 41 days in the second set indicates that the culture is a pure tetraploid.

*Experiment III.*—That the direction of the mutagenic action is independent of the duration of the treatment was confirmed by running a third batch. There were two alterations in the procedure. The treatment was carried out at a constant temperature and the control itself was a mixture at the start of the experiment (Photos 34 and 35). Spontaneous tetraploidy has been reported in the control strain (Prema Bai and Subramaniam, 1947). Confirmation for the above is offered by the tetraploid sectors in Photo 34. The rest of the population in the tube was a mixture of *Smooth I, Smooth III* and *Rough II* cells (Photos 34 and 35). Naturally, even after 6 days treatment the tetraploid cells predominate (Photos 36 and 37). Both these colonies show the persistence of the diploid types in the shape of knob-like growths in the centre. This picture persists even after 15 days of treatment when the colonies were of the tetraploid type (Photos 38 and 39). The presence of the tetraploid sectors in the control colony itself (Photo 34) at the commencement suggests the possibility that the predominance of the tetraploid cells on continued treatment with chrysene may merely be the result of relatively accelerated rate of growth of the tetraploid and that chrysene may have had nothing to do with it.

*Experiment IV.*—After the lapse of an year a fourth batch was run. The top yeast mutant obtained in 1945 by Subramaniam and Ranganathan (1946) did not appear under identical experimental conditions two years later (Subramaniam and Krishna Murthy, 1949). It has to be surmised that genic sequences may vary periodically. To test whether chrysene like acenaphthene (Subramaniam and Ranganathan, 1946) and cold shock (Subramaniam and Ranganathan, 1947; Ranganathan, 1950) may produce a top yeast along with a tetraploid, the present experiment was carried out. However, observations on the effect of chrysene which extended over a period of 18 months failed to reveal the occurrence of top yeast resulting from a chromosomal translocation or breakage.

At the commencement the control was a mixture of various types (Photos 40 and 41). There is a smooth, rapidly growing sector in photo 40, and the mixed sculpturing of the photos suggest that the control culture contains diverse types. As in the preceding observations, the smoother types predominate after 5 days of exposure to chrysene (Photo 42). The appearance of a regular, smooth outer border in the colony illustrated in Photo 42 suggested that the mutation may have occurred during the growth of the colony. After 10 days of treatment the *Rough I* types appear again. Photo 43 shows scattered thin streaks of *Rough II* cells, while in Photo 44 these form distinct sectors. This mixed nature of the various types persist

even after 16 days of treatment (Photos 45 and 46), and the tetraploid sectors appear only after 23 days of treatment, the quadruplicate colonies for which are given as photos 47, 48, 49 and 50. Photos 47 and 50 are typical tetraploids. *Rough II* cells appear as streaks in Photo 48 where the other regions are of *Smooth I* and *Smooth II*. A typical *Smooth I* colony with a very insignificant *Rough II* sector is illustrated in Photo 49.

## Discussion

There are numerous reports (Cook, Hart and Joly, 1939; Tolmatcheva, 1940; Dodge, Dodge and Johnson, 1941; Bauch, 1941 *a*, *b*, 1942 *a*, *b*, 1943; De Clerck, 1942) on the effects of known carcinogens and allied substances on yeast cells. Unfortunately, these are on strains of unknown chromosomal and genetic constitution and as such they have formed only interesting forerunners for more exact investigations. The problem of chemical mutagenesis has assumed an unusual importance only during the past one decade. The investigations on the effects of chemicals on yeasts were either carried out long before the importance of chemical mutagenesis was realised or did not take into account the probable genetic effect of the chemicals themselves. Naturally in spite of the interesting nature of the reports these are only of historical interest to us.

The results presented in this paper while confirming the mutagenic ability of chrysene suggests that the induction of polyploidy by the chemical is not an all-or-none reaction. Tetraploid sectors and tetraploid colonies were observed after different durations of exposure. As has been emphasised in a previous contribution from this laboratory (Subramaniam and Sreepathi Rao, 1950) on the effect of camphor on yeast the main fact that emerges is that chrysene has accelerated the rate of mutation but has no influence on the direction of the mutation. Neither does it form a selective environment for any particular mutant. On the basis of reverse mutations in yeasts it was shown by Subramaniam, *et al.* (1948) that multiple alleles exist at the locus governing the nature of sculpturing of the giant colonies. Chrysene accelerates the rate of mutation at the locus.

The spontaneous but sporadic occurrence of the tetraploid sectors in the diploid colonies suggests the possibility that even an induction of tetraploidy should be preceded by a gene mutation. Evidence for such a conclusion is available from experiments in different directions in this laboratory. By culturing the acenaphthene induced tetraploid, BY-3, in media containing a high percentage of alcohol, Duraiswami and Subramaniam (1950) could induce its reversal to the diploid condition. Though the strain, BY-3, was isolated in the year 1945 (Subramaniam 1945, 1947) its colony has never

shown any diploid sector during the past four years. It forms spores and the spores should have a balanced chromosome constitution. As is common in yeasts, many asci contain only a single spore. As such, the highly stable nature of the acenaphthene-induced tetraploid suggests that the spores should have a tendency to fuse with similar ones. If acenaphthene has merely duplicated the chromosome complement it is obvious that such a mechanism need not be present. The tendency for fusion, therefore, has arisen as a natural corollary to the duplication of the chromosome complement. The question arises as to the nature of the accompanying change rendering it imperative for the spores of the tetraploid to fuse. The recovery of the diploid by the long culturing of the tetraploid in medium containing a high percentage of alcohol (Duraiswami and Subramaniam, 1950) suggested that the change is reversible. As may be gathered from literature, gene mutations are reversible. The fact that the tetraploid sectors appear periodically in the diploid colony suggested that there should be a locus for polyploidy. Many chemicals inhibit cell division but only a few are capable of producing stable tetraploids. If, as the recovery of the diploid from the tetraploid indicated, a gene mutation should precede a duplication of the chromosome complement, the analysis of the factors governing the induction of polyploidy becomes interesting.

Subramaniam and Sreepathi Rao (1950) suggested that the main role played by chemicals in inducing tetraploidy is the induction of a specific mutation. Narcotics inhibit cell division and often produce temporarily tetraploid cells which revert to the diploid condition by multipolar mitoses. If the inhibition of the cell division with the resulting so-called ' C-mitosis ' (Levan and Sandwall, 1943; Levan and Ostergren, 1943; Dermen, 1940) is the main factor in producing stable tetraploids then it stands to reason that all agencies capable of inhibiting cell division should automatically produce tetraploidy. Why is it then that the action of colchicine is different from that of chloral hydrate (Kemp, 1910) ? It appears that the mere production of the so-called C-mitosis is actually unrelated to the doubling of the chromosome complement. The polyploidogens should induce a gene mutation enabling the duplicated complement of chromosomes to function harmoniously. If that is so, the polyploidogens should accelerate the rate of mutation at other loci as well. The results presented in this paper offer confirmation of the earlier demonstration (Subramaniam and Sreepathi Rao, 1950). If the spontaneous rate of mutation at the locus for polyploidy is comparatively low, then the appearance of tetraploid cells should show a much lower frequency in experiments with polyploidogens also. In other words, gene mutations at other loci may be much more common than the

tetraploid sectors in materials exposed to the action of known polyploidogens. As would be seen from the illustrations, gene mutations have been accelerated by chrysene. There is no selective action. It is this increased mutation rate, comparable to what Strong (1946, 1947) terms as an 'unstable genetic state' that is responsible for even an induction of polyploidy by chrysene.

There is just the possibility that, as claimed by Demerec (1948), certain chemicals may have a circumscribed and specific action. It may be remembered that dibenzanthracene produced only translocations (Demerec, 1948). Are the polyploidogens very specific in their action? The results recorded in the present paper indicate that any chemical which is a mutagen would also be a polyploidogen. Acceptance of this suggestion renders it possible to offer a new explanation as to why many chemicals inducing C-mitosis are not capable of producing the stabilizing gene mutation necessary for tetraploidy.

The photographs offer proof that the induction of polyploidy is determined by the duration of the treatment. As has been observed by Demerec (1948), the same length of exposure need not produce identical results. If a certain duration is necessary for the induction of the gene mutation preceding the doubling of the chromosome complement, only certain chemicals could be classified as polyploidogens.

The question of the toxicity of the chemical looms large in the picture. A toxic mutagen during a short exposure may induce mutations at these loci which have high mutation rates. When the spontaneous appearance of tetraploidy is comparatively rare, one has to assume that the mutation rate at the locus for polyploidy should be very low. Naturally, to produce a mutation at the specific locus a longer exposure to the mutagen would be necessary. When the substance is toxic, any such attempt would meet with a failure since the organism itself would die.

With the published results there is no reason to believe that colchicine and acenaphthene produce only very specific mutations. It has to be remembered that accelerating the rate of mutation at even a specific polyploidy locus in a diploid does not mean that only tetraploids can appear. As far back as 1938 Navashin obtained a haploid by the treatment of a diploid with acenaphthene. Judged on this basis, it is not surprising that chrysene which induces polyploidy should also induce gene mutations.

The dividing line between a mutagen and a carcinogen is rather tenuous. The earlier investigators had taken into consideration only gene mutations and chromosomal rearrangements. They do not seem to have considered

that carcinogens may induce polyploidy. If chrysene which induces polyploidy is also in general mutagenic, it appears quite likely that the known carcinogens may produce similar reactions. The picture is complicated by the differentiated nature of the mammalian tissue and also by the possible existence of specific genes not found in *Drosophila* or in yeasts. But if it could be shown that the potent carcinogens induce gene mutations, chromosomal rearrangements and polyploidy, the problem of carcinogenesis itself appears capable of being approached on a rational basis.


## Summary

1. An investigation on the effect of chrysene on yeast was planned with an idea to evaluate clearly the changes induced by carcinogens. An important angle of approach has been to evolve methods for the genetical improvement of industrially important micro-organisms.

2. Vegetative cells of the control diploid strain, BY-1, were exposed to the action of chrysene and the progressive changes undergone by the population as a result of the treatment was followed up by the observation of the giant colony characteristics at intervals.

3. Four independent series of experiments were conducted in the course of about an year the results of which have been illustrated with photographs.

4. An analysis of the changes in the population of mutants at the different stages of the treatment reveals that the chemical accelerates the rate but has no influence on the direction of the mutation. It does not seem to form a selective environment for any particular mutant.

5. The results presented while confirming the general mutagenic ability of chrysene suggest that the induction of polyploidy by the chemical is not an all-or-none reaction.

6. It appears that the mere production of the so-called C-mitosis is actually unrelated to the doubling of the chromosome complement. The polyploidogens should first produce a gene mutation enabling the duplicated chromosomes to function harmoniously.



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## DESCRIPTION OF THE PHOTOGRAPHS

All the colonies were grown in barley malt agar and at the room temperature. 15 ml. of the medium were used for each petri-dish (9·5 cm. in diameter).

| Expt. No. | Photo No. | Period of treatment in days | Period of growth in days | Diameter of the colony in cm. | Photographed on |
|---|---|---|---|---|---|
| I | 1 | Nil (BY 1, control) | 20 | 3·7 | 22–9–1948 |
| | 2 | ,, | ,, | 3·9 | ,, |
| | 3 | 1 | 18 | 3·6 | ,, |
| | 4 | ,, | ,, | 3·7 | ,, |
| | 5 | 5 | 15 | 3·2 | 23–9–1948 |
| | 6 | ,, | ,, | 2·5 | ,, |

| Expt. No. | Photo No. | Period of treatment in days | Period of growth in days | Diameter of the colony in cm. | Photographed on |
|---|---|---|---|---|---|
| | 7 | 13 | ,, | 3·3 | 1–10–1948 |
| | 8 | ,, | ,, | 3·2 | ,, |
| | 9 | ,, | ,, | 3·3 | ,, |
| | 10 | 21 | ,, | 2·9 | 9–10–1948 |
| | 11 | ,, | ,, | 2·9 | ,, |
| | 12 | ,, | ,, | 2·7 | ,, |
| | 13 | ,, | ,, | 2·6 | ,, |
| | 14 | 30 | 17 | 2·8 | 20–10–1948 |
| | 15 | ,, | ,, | 2·6 | ,, |
| | 16 | ,, | ,, | 3·2 | ,, |
| | 17 | ,, | ,, | 2·9 | ,, |
| | 18 | 39 | 14 | 2·3 | 26–10–1948 |
| | 19 | ,, | ,, | 2·5 | ,, |
| II | 20 | Nil (BY 1, *control*) | 13 | 3·3 | 24–5–1949 |
| | 21 | ,, | ,, | 3·3 | ,, |
| | 22 | 5 (in 1st set) | 16 | 3·7 | 2–6–1949 |
| | 23 | ,, | ,, | 3·7 | ,, |
| | 24 | 5 (in 2nd set) | ,, | 3·5 | ,, |
| | 25 | 12 (in 1st set) | 13 | 2·8 | 6–6–1949 |
| | 26 | ,, | ,, | 3·8 | ,, |
| | 27 | 12 (in 2nd set) | ,, | 3·7 | ,, |
| | 28 | 18 (in 1st set) | 10 | 3·0 | 10–6–1949 |
| | 29 | 18 (in 2nd set) | ,, | 2·9 | ,, |
| | 30 | 28 (in 1st set) | 9 | 2·8 | 18–6–1949 |
| | 31 | 34 (in 1st set) | 8 | 2·7 | 23–6–1949 |
| | 32 | 34 (in 2nd set) | ,, | 2·7 | ,, |
| | 33 | 41 (in 2nd set) | 10 | 2·3 | 2–7–1949 |
| III | 34 | Nil (BY 1, control) | 13 | 2·6 | 18–7–1949 |
| | 35 | ,, | ,, | 2·2 | ,, |
| | 36 | 6 | ,, | 2·3 | 25–7–1949 |
| | 37 | ,, | ,, | 2·5 | ,, |
| | 38 | 15 | ,, | 2·5 | 3–8–1949 |
| | 39 | ,, | ,, | 2·4 | ,, |
| IV | 40 | Nil (BY-1, control) | 19 | 2·1 | 6–10–1949 |
| | 41 | ,, | ,, | 2·1 | ,, |
| | 42 | 5 | 14 | 2·4 | 7–10–1949 |
| | 43 | 10 | 13 | 2·4 | 10–10–1949 |
| | 44 | ,, | ,, | 2·4 | ,, |
| | 45 | 17 | 14 | 3·1 | 19–10–1949 |
| | 46 | ,, | ,, | 2·8 | ,, |
| | 47 | 23 | 17 | 2·3 | 28–10–1949 |
| | 48 | ,, | ,, | 2·7 | ,, |
| | 49 | ,, | ,, | 2·5 | ,, |
| | 50 | ,, | ,, | 2·0 | ,, |

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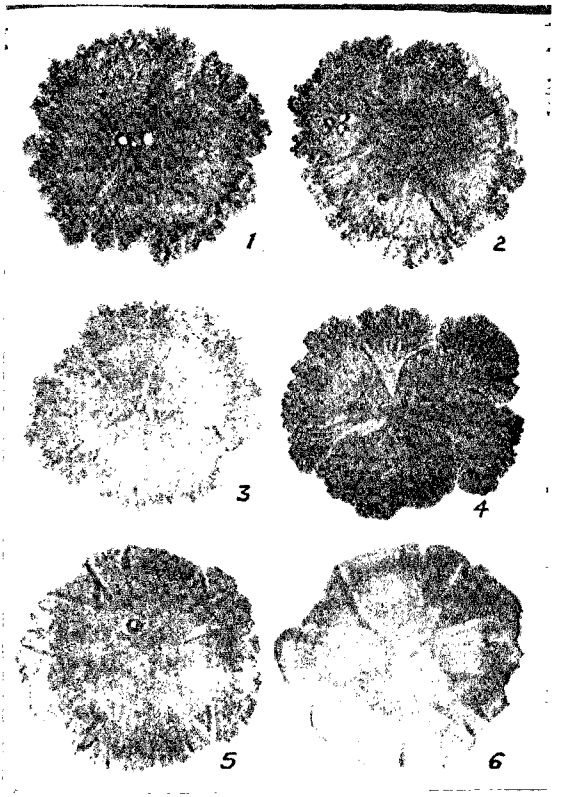
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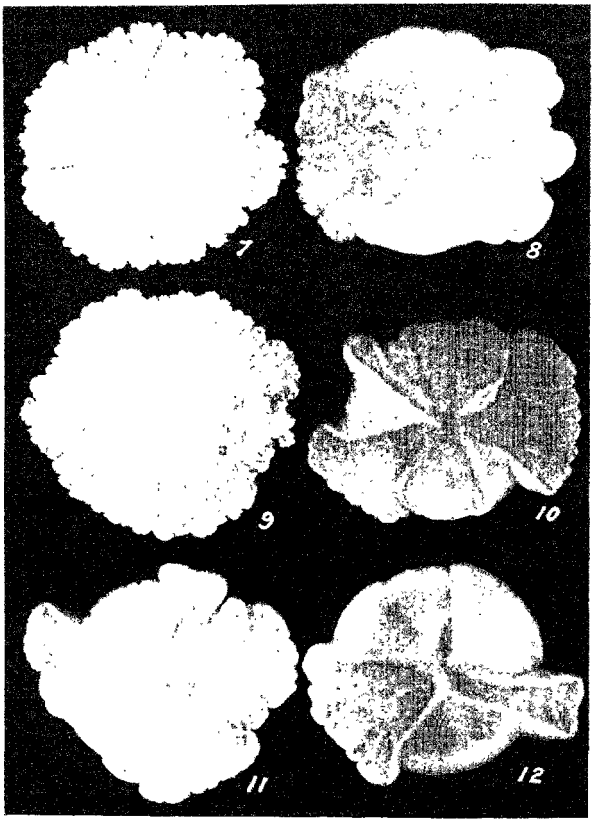
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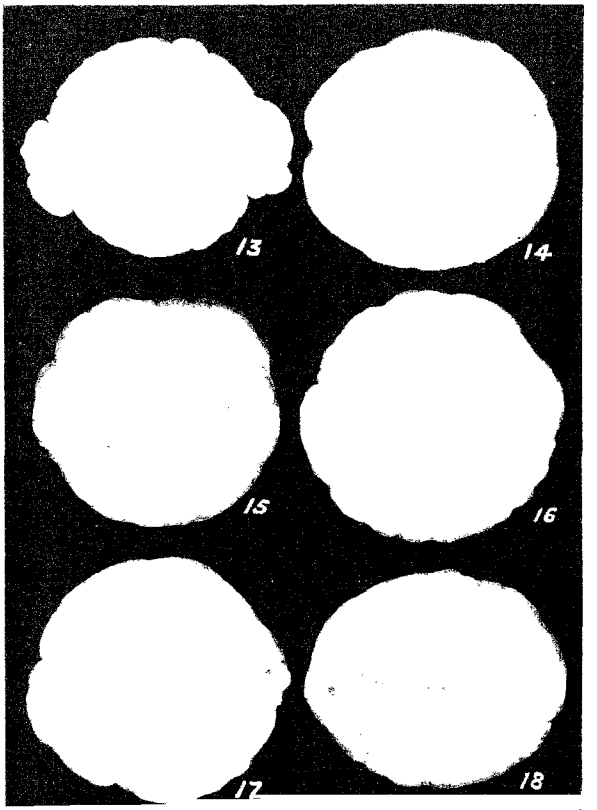
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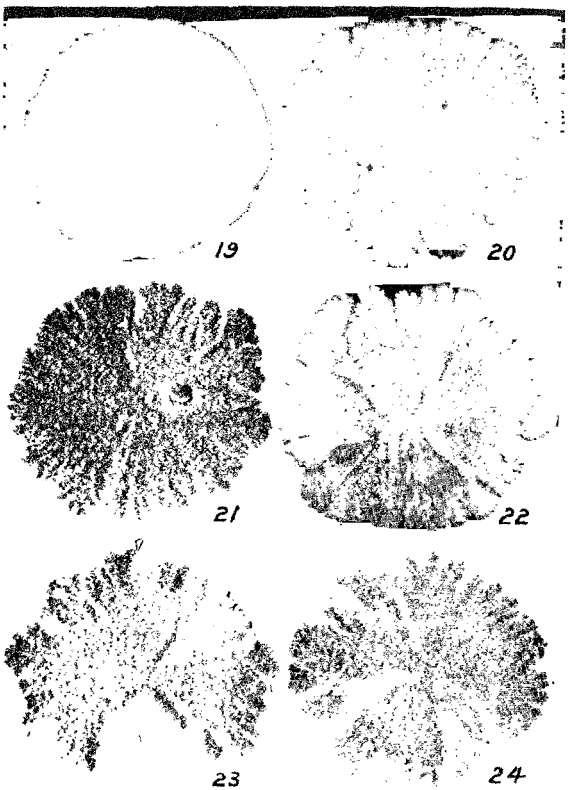
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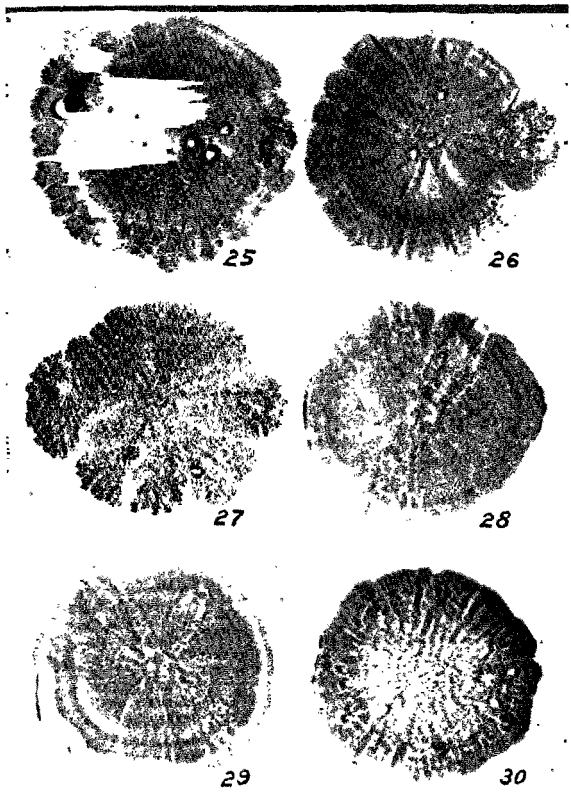
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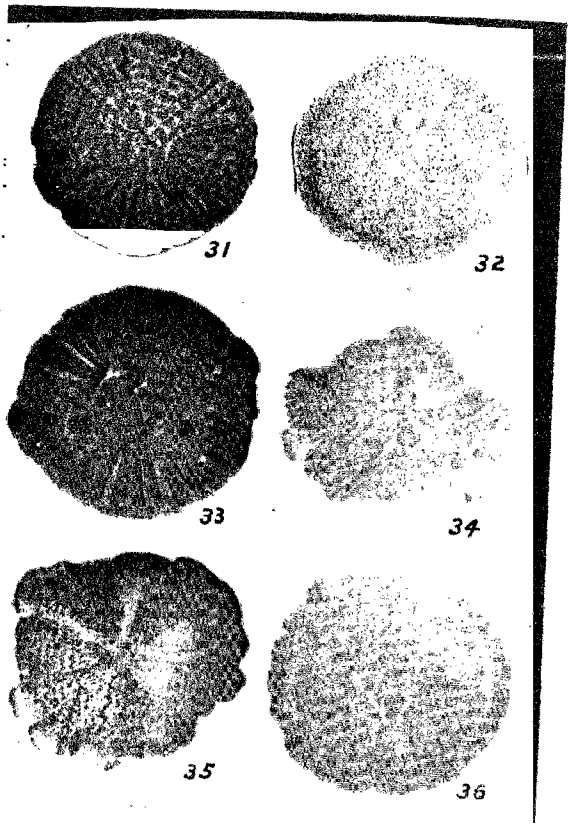
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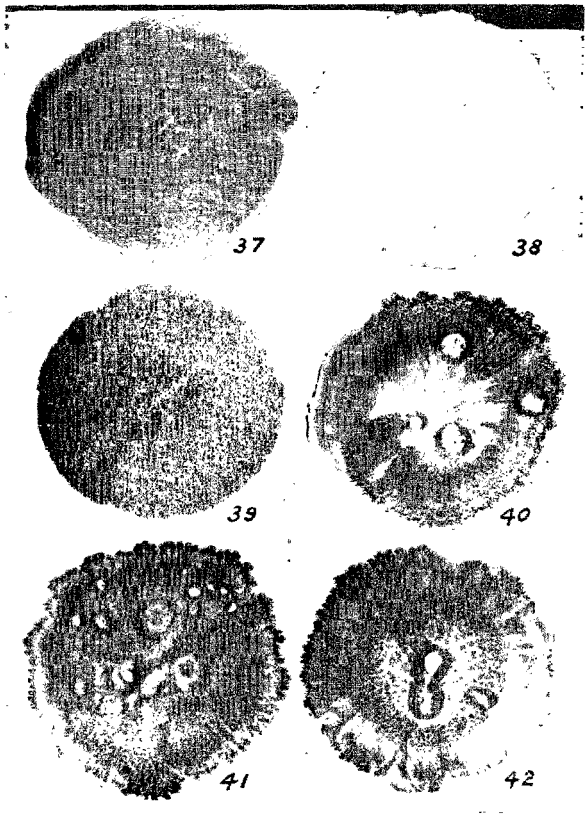
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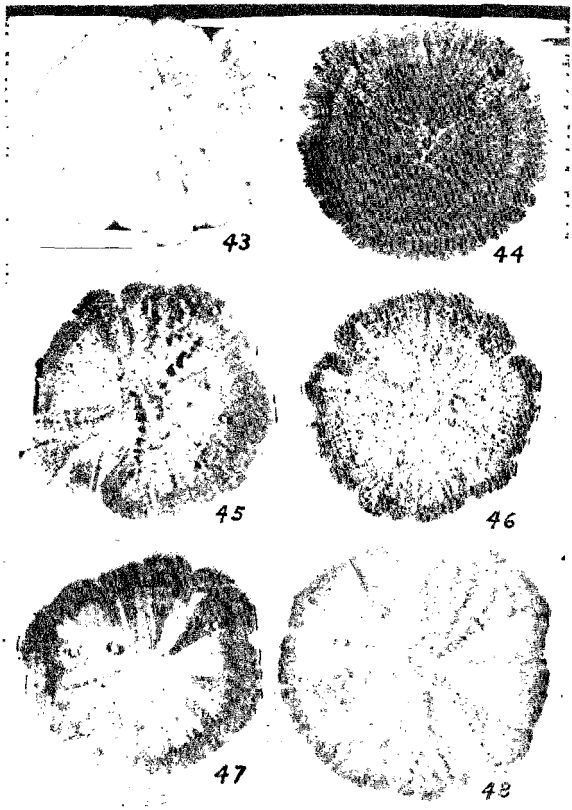
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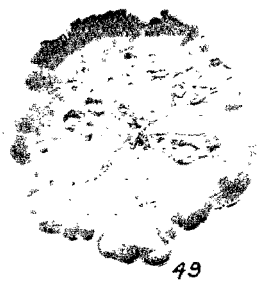
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