STUDIES ON THE MUTAGENIC ACTION OF CHEMICAL AND PHYSICAL AGENCIES ON YEASTS

IV. The Effect of Camphor on Fermenting Yeast Cultures

BY M. K. SUBRAMANIAM, M.A., D.Sc. AND S. K. SREEPATHI RAO, B.Sc.

(*Cytogenetics Laboratory, Department of Biochemistry, Indian Institute of Science, Bangalore* 3)

Received October 24, 1950

(Communicated by Dr. K. V. Giri, F.A.SC.)

CONTENTS

INTRODUCTION

FIVE years back, a tetraploid was isolated after treatment of our two chromosome control strain of brewery yeast, BY 1, with acenaphthene (Subramaniam, 1945, 1946). This identification of the tetraploid was based entirely on cytological data (Subramaniam, 1947). A detailed investigation of a naturally occurring tetraploid distillery yeast (Subramaniam and Ranganathan, 1946; Ranganathan and Subramaniam, 1948) led to the suspicion that polyploidy may be more common in yeasts than imagined. This was confirmed by the spontaneous but sporadic occurrence of tetraploidy which has been repeatedly observed in our two chromosome control strain (Prema Bai and Subramaniam, 1947; Subramaniam, Ranganathan and Krishna Murthy, 1948; Subramaniam and Krishna Murthy, 1949). The earlier

induction of tetraploidy with acenaphthene was, therefore, not at all surprising. An extensive series of investigations indicated that it is easy to duplicate the chromosome complement by physical and chemical agencies (Subramaniam and Ranganathan, 1948, 1949; Subramaniam and Krishna Murthy, 1949; Mitra and Subramaniam, 1949; Ranganathan and Subramaniam, 1950). The tetraploids obtained after treatment with different agencies have been kept under observation for the past three years. They are highly stable (Duraiswami and Subramaniam, 1950). The facility with which a doubling of the chromosome complement could be achieved in this laboratory posed the question as to why most of the previous investigators failed to isolate stable tetraploids ? This necessitated a planned series of investigations.

The reasons for their failure were obvious: (*a*) They did not know whether the strains used by them in their experiments were " haploid ", " diploid " or " polyploid ". (*b*) They arbitrarily classified yeasts on purely morphological criteria into the so-called " haploid " and " diploid " types on the assumption that polyploidy does not occur in yeasts. This classification rendered it impossible for them to identify even those polyploids which might have originated in their experiments. (*c*) They were not in a position to distinguish polyploidy from chromosomal translocations and either of them from gene mutations. (*d*) Naturally, they could not critically evaluate the effect of the various agencies on yeast strains of differing genic and chromosomal constitutions. (*e*) Since they were not aware of the fundamental cytological changes that take place during the shift from the aerobic to the anaerobic type of metabolism, their methods of approach to the problem appear to be not conducive to the successful isolation of polyploids.

Since under specified conditions tetraploids have been isolated, it has become possible to demonstrate that the very short exposure to the polyploidogens by the earlier investigators might not have led to an induction of polyploidy assuming for the time being that they were experimenting with a diploid strain.

CAUSES FOR CONFUSION

Winge and Roberts (1948) remark that they did not consider " a parallel cytological study worthy of undertaking " (p. 311). The reasons adduced are that yeast chromosomes are very difficult to observe and that there are considerable divergences of opinion between the investigators. The observations are interpreted on the belief that the starting strains are " diploids " and the segregations observed are explained on a series of assumptions.

Since polyploidy is common it appears that even their initial assumption that the starting strains are diploids may be questionable (Subramaniam, 1950, *a*, *b*, *c*). If yeast cytology is in a confused state it stands to reason that a clarification of this confusion should precede and not succeed genetical investigations.

Skovsted (1948) observes that his experiments " have not supplied any evidence in favour of Bauch's explanation that camphor is a chemical which produces polyploids ". He continues: " A direct proof has not been possible so far as yeast has been a very unsatisfactory object cytologically " (p. 260). The aim of his experiments was to investigate whether camphor had any general effect on the mutability of yeast. Treatment for 24 hours was found to induce the formation of sectors in giant colonies and of abnormal dwarf colonies. He concludes that camphor treatment " induced the mutability and that the camphor cells may deviate genetically from the cells of normal appearance " (p. 253). Treatment for 48 hours further increased the mutability. In experiments with a brewer's yeast he found that some of the mutants which appeared in the camphor material were new and not observed before. " The general increase in mutability " is stated to be " twice or three times the spontaneous rate " and it is considered impossible " to explain the camphor mutants as rare spontaneous mutants " (p. 259). To the simple question whether these " mutants " are the result of (*a*) polyploidy, (*b*) polysomy, (*c*) chromosomal translocations or (*d*) gene mutations, there is no answer in the paper. While suggesting that no evidence was obtained for any belief that the camphor mutants may be " directed mutations " he considers that the much greater range of variation observed in the " diploid brewer's yeast ", may be explained on the basis of cytological irregularities due to instability. When we remember that the chromosome constitution of the strains experimented on were unknown, the possible validity of the speculation could be just imagined.

The basis for the above suggestion is Levan's (1947) assertion that yeast should have at least ten chromosomes. Levan's (1947) claim itself is based on apparently wrong premises. He does not seem to have considered the possibility that the cytology of cells actively multiplying aerobically may be different from those in active fermentation. *All his investigations were carried out on fermenting cells.* Our investigations show that the cells become endopolyploid during fermentation (*Subramaniam*, 1947, 1948 *b*). The cytological pictures presented by Levan are therefore those of endopolyploid cells. It is remarkable that the curious pictures observed by him were illustrated by us (Subramaniam, 1948 *b*) in fermenting cells stimulated

to divide by the addition of fresh wort. *But what must be remembered is that the same strain during the aerobic phase shows only two chromosomes* (Subramaniam, 1946). Fermenting cultures contain a small percentage of mitotically dividing cells showing the normal two chromosomes. Since Levan never investigated the cytology of his strain during the aerobic phase, and since fermenting cells are endopolyploid, the actual chromosome number should be considerably smaller than that illustrated by him in his plates. It is not customary to generalize about the number, structure and size of chromosomes from endopolyploid cells. Just because liver cells show amitosis-like phenomena (Kater, 1940), it does not entitle one to suggest that similar phenomena should occur in all cells. Neither do investigators generalize about the shape, size and structure of the chromosomes in all tissues of *Drosophila* merely from a study of the salivary chromosomes. Under the circumstances the validity of Levan's claim that yeasts should have at least ten chromosomes appears questionable. His criticism of the claim of the other workers who reported a lower chromosome number is rather unfortunate. It implies that all yeast strains should have only ten chromosomes and Skovsted (1948) seems to have proceeded on that assumption. It has to be distinctly understood that the various investigators (Badian, 1937; Sinoto and Yuasa, 1941; Skovsted, 1948; Bauch, 1941; Levan, 1947) have been experimenting with different strains. It is too much to expect that all strains of yeasts, whatever their source should have a minimum number of ten chromosomes. Levan (1947) admits that polyploid yeasts should have originated during the investigations of other workers. If that is so, spontaneous polyploidy should be common and yeast strains having different chromosome numbers should occur. The minimum number of chromosomes possible in a diploid strain is two and our control strain, BY 1, has that number (Subramaniam, 1946). From the fact that centrioles with centrospheres were demonstrated (Ranganathan and Subramaniam, 1947) we have no doubt that our technique of handling yeasts for cytological investigations (Subramaniam, 1948 *a*) gives perfect pictures. It is true that fermenting cells when induced to divide show chromosome fusions (Subramaniam, 1948 *b*). But fermenting cells like glandular cells die and disintegrate after varying periods of activity. If we consider the macronucleus of Ciliates to be endopolyploid (Subramaniam, 1947) then, we have to believe that even endopolyploid nuclei can show limited powers of division. It is stated by Sonneborn (1947) that the macronucleus may divide over a hundred times before it is replaced by a new one. Similarly the fermenting cells may be capable of a few divisions and the endopolyploid nuclei of such cells may even show amitosis. This is probably the reason

for Guilliermond's (1920) claim that the yeast nucleus divides amitotically. But there is an important point to be remembered in this connection. As in gland cells, the final fate of fermenting cells is death and disintegration.

Levan and Sandwall (1943), Levan (1947) and Skovsted (1948) seem to have studied the effect of camphor on fermenting cells. As has been shown in a previous contribution (Subramaniam, 1948 *b*) fermenting cultures contain a small percentage of mitotically dividing cells. The various mutants observed by Skovsted (1948) should be the result of the action of camphor on these few mitotically dividing cells. As will be shown in the discussion, the " camphor forms " may be the result of the action of camphor on endopolyploid cells. A critical study reveals that even if some of the " mutants " observed by Skovsted (1948) were polyploids, he could not have identified them purely on morphological criteria. Realizing this difficulty he remarks: " Thus if camphor treatment produced polyploids as suggested by Bauch, the new types would become diploid, a process which is much easier to confirm on morphological character than the change from diploid to tetraploid " (p. 250). His experiments were, therefore, carried out mainly on a so-called " haploid " strain of yeast isolated by Winge and Laustsen. The question naturally arises whether the strain was, after all, a haploid (Subramaniam, 1950 *a*).

Winge and Laustsen (1937) differentiated their so-called " haploids " from " diploids " not on cytological grounds but on the basis of morphology. Cells arising by the direct germination of a spore were identified as " haploids ". Such strains were asporogenous and said to be composed of small round cells showing " short shoot " growth. Sporogenous cultures originating by spore, cell or nuclear fusion and composed of long oval cells showing " long shoot " growth were classified as " diploids ". It is known that yeasts are polymorphic (Guilliermond, 1920) and hence the only character on which some reliance could be placed is the ability to form spores. The strain used by Skovsted (1948) is illustrated by Winge and Laustsen (1937) as photo 21. But they illustrate as photo 20 in the same paper, a diploid which did not form spores. The identification of that asporogenic strain as a diploid should have been made obviously on size, shape and mode of budding of the cells. But then, Winge (1935) considers that his so-called " haploids " may occasionally show " long shoot " growth (pp. 95 and 98). Thus we are left only with shape and size of the cells and all investigators on yeasts know that these are highly variable. Skovsted's (1948) claim that it is easy to differentiate a " haploid " from a " diploid " purely on morphology has very little experimental justification,

Granting that his so-called " haploid " was a real haploid how can one be sure that if camphor treatment duplicated the chromosome complement, the resulting diploid would form spores? Winge and Laustsen (1937) suggest that the asporogenous character of their so-called " diploid " (Pl. VI, Fig. 20) may be the result of an inbreeding degeneration, but in their paper on inbreeding degeneration (1940) they suggest only that the " degeneration reveals itself in a perceptible reduction of the germination percentage of the spores " (p. 37). Assuming for the time being that some of the homozygous types may be asporogenous (Winge and Laustsen, 1937) such remarkable degeneration was observed only after " direct diploidization ". A doubling of the chromosome complement if induced by camphor is strictly comparable to the so-called " direct diploidization ". The " haploid " used by Skovsted (1948) in his camphor experiments was isolated by Winge and Laustsen (1937) from *that ascus, a spore from which produced an asporogenous diploid.* There was no logical reason to believe that if the so-called " haploid " had been converted into a " diploid " it should have produced spores. It may be asporogenous like the other so-called diploid which arose from a sister spore.

The conclusion is obvious. It is not easy to confirm on morphological character alone a change from " haploidy " to " diploidy " as imagined by Skovsted. A simple experiment carried out some three years ago (Subramaniam and Krishna Murthy, 1949) confirmed the conviction that morphological characters unsupported by cytological evidence are valueless for any correct evaluation of the probable genetical changes induced by polyploidogens. There is a distillery yeast, DY 1, in our collection which is a tetraploid (Subramaniam and Ranganathan, 1946; Ranganathan and Subramaniam, 1948). It produces a smooth giant colony indistinguishable from that of the autotetraploid brewery yeast, BY 3. Actively growing cells of the distillery strain were treated for an year with acenaphthene. From the treated culture top and bottom yeasts were isolated. The giant colony of the top yeast was indistinguishable from that obtained after treatment of the two chromosome brewery strain with acenaphthene, while that of the bottom strain was entirely similar to that of the control distillery yeast at the commencement of the experiment. The fact that treatment for one year produced a top-yeast indicated that acenaphthene had some action even on the tetraploid distillery yeast. The remarkable similarity of the sculpturing of the top yeast obtained from the distillery strain to that isolated from the brewery yeast does not entitle one to deduce without cytological confirmation that both have comparable chromosome constitutions. For, it has to be remembered that the control strains from which

they were obtained had entirely different chromosome constitutions. Similarly, just because the giant colony of the bottom strain isolated after treatment for one year with acenaphthene is identical in appearance with that of the control tetraploid strain, it does not follow that acenaphthene has not induced a chromosomal duplication. Such an assertion would be possible only if cytological investigations confirm that giant colonies of octoploids are entirely different in appearance from that of tetraploids.

When that is the situation in experiments where the chromosome constitution of the control strains are known, one can imagine the confusion where experiments are carried out on diverse strains of unknown chromosome constitution. This indicated that for orderly advance, investigations should be carried out on a strain whose cytological and genetical changes had been carefully followed up over a period of time.

Criteria for the Identification of Tetraploids

Cytological investigations on yeasts are time-consuming and hence simpler methods for the differentiation of tetraploids from diploids were devised. The giant colonies of the strains isolated after treatment of the two chromosome control brewery yeast with acenaphthene were remarkably similar to those obtained after culturing it in the ice-room (Subramaniam and Ranganathan, 1948). It was surmised that the characteristics of the giant colonies could be employed for the identification of the tetraploids obtained by treatment of our control strain. It was discovered (Subramaniam, 1947) that after a single duplication of the chromosomes, acenaphthene had no further action on the cells. Simultaneous investigations on the cytology and giant colony characteristics of the acenaphthene induced tetraploid, BY 3, during a period of 24 months showed that the highly stable nature of the giant colony characteristics of the autotetraploid (Subramaniam and Krishna Murthy, 1949; Duraiswami and Subramaniam, 1950) could be used to identify any induction of polyploidy. Unlike the tetraploid, the two chromosome control strain showed a variety of mutants during the same period (Subramaniam, Ranganathan and Krishna Murthy, 1948; Krishna Murthy and Subramaniam, 1950; Mallya and Subramaniam, 1949). Duraiswami and Subramaniam (1950) were able to recover the diploid from the autotetraploid, BY 3, and the recovered diploid showed a corresponding seasonal variation in the predominance of the different mutants like the two chromosome control. Mitra and Subramaniam (1949) again confirmed the validity of the use of giant colony characteristics for the identification of autotetraploids by parallel cytological investigations. Chrysene, like

acenaphthene, produces only a single duplication of the chromosome complement even when the culture was treated for 40 days.

Under the circumstances it appears that giant colony characteristics alone could be employed to locate and distinguish the tetraploids induced by different agencies in our two chromosome control brewery strain.

PLAN OF THE EXPERIMENT, MATERIAL AND METHODS

In experiments on the effect of polyploidogens on fermenting yeast cultures, the effect of the chemical on the endopolyploid cells have to be clearly differentiated from those on the small percentage of mitotically dividing cells. It has been shown elsewhere (Subramaniam, 1948 *b*) that a five-day old fermenting culture contains only a small percentage of mitotically dividing cells. When a loop of material from an aerobic culture is inoculated into a tube of fresh wort, these show initially a logarithmic growth phase (Prema Bai and Subramaniam, 1947; Mallya and Subramaniam, 1949). The end of the logarithmic growth phase probably coincides with the depletion of the available oxygen. Even during the logarithmic growth phase, a slowly increasing percentage of cells should have become endopolyploid. At the end of the logarithmic growth phase when the population shows little fluctuation, the majority of the cells probably become endopolyploid. The second cycle of growth observed in our control, BY 1, is apparently the result of the division of these endopolyploid cells. There is nothing surprising in such a possibility since the macronucleus of Ciliates (Sonneborn, 1947; Subramaniam, 1947) divides normally by amitosis.

If the inoculum introduced is heavy, the transformation to the endopolyploid fermenting condition is naturally quicker. With the progress of fermentation only a very small percentage of cells, comparable to the replacement cells in glandular tissue would retain their ability to divide mitotically. If the inoculum is a mixture of gene mutants, the relative viabilities of the different mutants would determine as to which of them would persist and continue to proliferate in a seven-day old fermenting culture.

All these different changes occurring normally in a fermenting culture have been taken into consideration in evaluating the results of the action of polyploidogens. When a loop of material from an aerobically growing culture is introduced into wort containing any polyploidogen, it is the mitotically dividing cells that are exposed to the action of the chemical during the logarithmic growth phase. As happens in most cases, if the drug induces only a single duplication of the chromosome complement and that quickly, the population at the end of the logarithmic growth phase would

consist of a mixture of diploid and tetraploid cells. The observations of Mitra and Subramaniam (1950) suggest that the effect of the polyploidogens cannot be characterized as an all or nothing reaction. Therefore, during the logarithmic growth phase a duplication of the chromosome complement in some cells may or may not occur. The chemical may induce gene mutations. A giant colony inoculation at the end of the logarithmic growth phase would give an idea of the nature of the changes that have taken place. Our cytological observations indicate that autotetraploids show a greater tendency to become endopolyploid owing to the quickened rate of their metabolism (Prema Bai and Subramaniam, 1947; Mitra, 1948). If the induced tetraploids become endopolyploid during the later stages of the logarithmic phase itself, they may be completely missed owing to their non-viability.

The cells present in a fermenting culture are of two types. The majority are endopolyploid and only a very small percentage retain their ability for unlimited proliferation. The effect of the polyploidogens incorporated in the medium ought to produce different results on these two categories of cells. Normally, endopolyploid cells are non-viable. Therefore a doubling of the chromosome complement in some of the small percentage of normal cells in a fermenting culture may make the population of such normal cells heterogeneous. Naturally, it should be possible to isolate tetraploids even from fermenting cultures, if they have not become endopolyploid. When giant colony inoculations are carried out from fermenting cultures, it has to be clearly understood that the resultant growth is by the small percentage of normal cells retaining their ability for unlimited proliferation. At every stage of testing the giant colony characteristics of the culture undergoing treatment with camphor, controls were run using material grown for 16 hours in wort.

The effect of synthetic camphor (Eastman Kodak) was tested on our well investigated two chromosome brewery strain, BY 1. A preliminary experiment planned with a view to estimate the optimum quantity to be added in order to get a good crop indicated that camphor induces gene mutations (Subramaniam and Sreepathi Rao, 1950). In order to obtain comparable results 2 gm. of camphor was dissolved in 5 ml. of 50% alcohol (Skovsted, 1948). Tubes containing one, two, three and four drops of the above solution of camphor incorporated in 5 ml. of wort were inoculated with a few cells from an aerobically growing culture of the control, BY 1. Giant colony inoculations were carried out 24, 48, 72 and 168 hours after the commencement of the experiments without any fresh addition of camphor. Such a series was expected to give an idea of the changes not only at the end of the logarithmic growth phase, but also after varying periods of fermenta-

tion. The giant colonies were grown in petri dishes of 7 cm. diameter on wort agar (Subramaniam and Ranganathan, 1948).

In the second series, the normal changes in the population of the control growing on an agar slant were investigated side by side with those induced by camphor. Initially an agar slant was inoculated with the control. After growth for 48 hours a loop of cells from the streak was introduced into wort and giant colony inoculations were carried out from such 16 hour cultures. Simultaneously one loop of material from the above liquid culture was introduced into each of the tubes incorporated with varying quantities of camphor. Every inoculation for giant colony from the culture undergoing treatment with camphor was followed by that of a sample from the agar slant culture grown in wort for 16 hours.

A few details regarding the procedure adopted for obtaining a correct picture of the population in a culture may not be out of place here. Not only is the inoculation carried out inside a sterile chamber, but the inoculated petri-dishes themselves are stored in the chamber. During some seasons the water vapour condensing on the surface of the medium used to result in a spreading of the colony. To obviate the difficulty, the wort agar was poured into the petri-dish only when the water-bath had cooled to 50° C. If in spite of this precaution, large droplets of condensed moisture occur on the inside surface of the cover of the petri-dish, the cover itself is replaced by a fresh sterile one. The inoculation is carried out only an hour or two after the setting of the medium. The nichrome needle used for inoculation is sterilized every time by repeated immersion in rectified spirit and burning off the alcohol. It has been found that if the tip of the needle is dipped into a well-shaken culture and then brought into contact with the dry inside wall of the test tube, uniform droplets could be obtained. The petri-dish is opened only just before inoculation and care is taken to see that the needle touches the surface of the medium but does not break it. If the surface of the medium is broken during inoculation, the growing colony assumes a slightly different appearance. It has to be emphasized that the small droplet at the tip of the needle would contain only a few yeast cells. When the culture is pure for a single type, duplicates would give identical types of colonies. But when the culture is a mixture of mutants, the few cells in the droplet used for inoculation may or may not contain a representative collection. Naturally, a number of inoculations have to be carried out to get a correct picture. Even when a single droplet contains all the different mutants, the giant colony developing may show two distinct categories of sculpturing. During some seasons, when two mutants appear to have an

almost identical growth rate at the same temperature, the sculpturing would be peculiar. That this result is due to a mixture of mutants could be made out by purifying the cultures by plating and testing the giant colonies developing from each of them. The above phenomenon is not so common. Usually, the mutants form sectors showing their characteristic configurations. All these factors which have been discovered as a result of investigations during the course of the past five years have been taken into consideration in evaluating the results obtained by the treatment of our control two chromosome strain with camphor. The classification of the various types of sculpturing is that given by Subramaniam, Ranganathan and Krishna Murthy (1948).

Observations

First Series.—The colony of the control inoculated on the 6th of August 1949 is illustrated in photo 1. It appears to belong to the *Rough II* category. On the 9th August, a loop from an aerobically growing culture of the control was inoculated into wort tubes containing one and two drops of a solution of camphor in absolute alcohol. Since at the end of 24 hours there was a good crop in the tube containing one drop of the camphor solution, a loop from the culture was inoculated into fresh wort. Giant colony inoculations were carried out from the above after growth for 16 hours. Photos 2 and 3 illustrate the colonies which developed. Both these show sectors. In photo 2 there are three *Smooth I* sectors and the sculpturing in the other regions of the colony is reminiscent of that produced by a mixture of *Smooth III* and *Rough I* types. This observation indicated the probability that camphor should have induced the following gene mutations.

Rough II $\begin{cases} \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Smooth} \\ \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Smooth} \end{cases}$ Smooth I

Rough II $\begin{cases} \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Smooth} \\ \text{Rough} \longrightarrow \text{Rough} \end{cases}$ Rough I

Rough II $\begin{cases} \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Lace} \\ \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Lace} \end{cases}$ Smooth III

Photo 3 reminds one of the *Rough* colony with a rim (*cf.*, photo 24, Krishna Murthy and Subramaniam, 1950) observed occasionally in the cultures of the control. Unlike the control (photo 1) which is homozygous for the *Rough* gene, the main part of the colony in photo 3 is heterozygous having the probable genic constitution *Rough/Rim.* It could have originated as a result of a single gene mutation.

Rough II $\begin{cases} \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Rim} \\ \text{Rough} \longrightarrow \text{Rough} \end{cases}$ Rough III

This colony (photo 3) also shows *Smooth I* and *Smooth II* sectors. The colonies directly inoculated from the 48 hours camphor culture are illustrated as photos 4 and 5. Since they are not identical, the culture is apparently a mixture of various mutants. The colony in photo 4 appears to have the genic constitution *Rough/Rim* while that in photo 5 is a mixture of *Smooth* and *Rough* types, the former predominating. The only reasonable possibility seems to be that camphor had induced gene mutations in different directions. With a view to confirm this remarkable result, a second experiment was carried out.

Control at the start of the experiment	Cultured in wort with one drop of camphor	
	After 24 hours	After 48 hours
Rough II	Rough I, Smooth III, Smooth I } Photo 2	Rough III Photo 4
	Rough III, Smooth I, Smooth II } Photo 3	Smooth I, Smooth III, Rough I } Photo 5

Second Series.—On the 23rd of August 1949, giant colony inoculations were carried out from the active culture of the two chromosome control prior to inoculation of loops of material into wort tubes containing one, two, three, four and five drops of camphor of the same concentration as used by Skovsted (1948). The control colonies after growth for 16 days are illustrated as photos 6 and 7. Both appear to belong to the *Rough II* category and both show *Smooth II* sectors at the periphery indicating that spontaneous mutations should have occurred during the growth of the colony. Twenty-four hours after inoculation there was growth only in the tube containing one drop of camphor solution. Therefore, simultaneous giant colony inoculations of the control and the culture growing in the presence of one drop of camphor solution were carried out. The control colonies were grown to eliminate the suspicion that the changes seen in the camphor culture are merely the normal mutations appearing in the control. The colonies of the control (photos 8 and 9) reveal that the culture had become a mixture during the interval. It should be remembered that the control was kept growing on the agar slant and that during growth there may occur changes in the populations of the mutants. Photo 8 shows that the nature of sculpturing of the major portion of the colony is due to a mixture of mutants. There is also a tetraploid sector. The duplicate presented as photo 9 is of the *Rough II* type. The colonies obtained by direct inocu-

lation from the culture which had grown for 24 hours in the presence of one drop of camphor are included as photos 10 and 11. Except for a few *Smooth* sectors which appear to have originated during the growth of the colony in photo 10, it could be classified as *Rough II* in spite of the fact that the granules are not so prominent. On the other hand, there are sectors of *Smooth II* in the colony shown in photo 11. The culture at the commencement of treatment was of the *Rough II* type and ignoring the *Smooth I* sector in photo 11, the following mutations should have occurred in the camphor culture:—

$$\text{Rough II} \begin{cases} \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Lace} \\ \text{Rough} \rightarrow \text{Mutation} \rightarrow \text{Smooth} \end{cases} \text{Smooth II}$$

A comparison of the photographs of the control (photos 8 and 9) with that of the 24-hour camphor cultures (photos 10 and 11) would indicate that the changes observed as a result of camphor treatment are in a different direction. The *Smooth II* type which dominates the colony in photo 11 is absent in photos 8 and 9. At the end of growth for 48 hours in the same medium, the population appears to be predominantly of the *Smooth II* type (photo 12). But this picture did not persist long. Colonies inoculated after treatment with one drop of camphor solution for 72 hours had reverted to the *Rough II* condition (photo 13). The control run at the same time (photo 14) was mainly of the *Rough II* type with *Smooth II* sectors. It is quite likely that the acceleration of mutability may have persisted even after removal from the medium containing camphor and during growth of the giant colony as evidenced by the Smooth sectors in photo 13. Preponderance of the *Rough II* cells in the control is nothing surprising, in view of the fact that two days previously (photos 8 and 9) the *Rough II* cells were dominating the picture. The change in the camphor culture is in another direction. The *Smooth II* cells which appeared in the 24-hour camphor culture (photo 11) and as extensive sectors in Photo 12, seem to have completely disappeared. On the 30th of August 1949, the population in the control (photo 15) was of the pure *Rough II* type reminiscent of the condition at the start of the experiment (photos 6 and 7). The duplicates from the 168 hour camphor culture show a mixture of mutants. In photo 16, the colony displays *Smooth I*, *Smooth II*, *Smooth III*, *Rough II* and tetraploid sectors. The colony in photo 17 on the other hand shows a rare mutant observed sporadically in the control (*cf.*, photo 13, Subramaniam, Ranganathan and Krishna Murthy, 1948). Rapidly growing tetraploid sectors in photo 17 are characteristic (*cf.*, photo 16).

In wort containing two drops of the camphor solution, there was growth only on the 6th day. That the culture had become a mixture of mutants

could be observed from photos 18 and 19. On the 8th day this mixed nature of the culture persisted and the colonies gave one the impression (photos 20 and 21) that the *Rough II* cells were predominating. On the 13th day after inoculation also, the culture retained the mixed nature of its population (photos 22 and 23). The surprising fact was that tetraploid cells had begun to appear as a result of treatment (sector in photo 23). In the cultures growing in wort containing either one (photo 16) or two drops of the camphor solution, tetraploid sectors are observed only sporadically. Duplicate control colonies (photos 24 and 25) run at the same time as photos 22 and 23 offer evidence that the control in the agar slant itself has become a mixture of *Rough II*, *Smooth II* and *Smooth I* types. It would be noticed however, that the changes in the culture of the control and that growing in the presence of one or two drops of camphor are not parallel.

DISCUSSION

Effect of Polyploidogens on Diploids and Tetraploids.—A survey of the literature reveals that after the initial enthusiasm regarding the possibilities opened up by the early experiments with polyploidogens, there has been a depression. It does not seem to have been realised that some of the plants treated with colchicine are natural polyploids whose genic and chromosomal constitutions are the results of conscious and unconscious selection. A perusal of the table given by Krythe and Wellensieck (1942) would indicate that many of the plants treated are themselves polyploids though the tabulation gives one the impression that these have been assumed for purposes of evaluation of the results of colchicine treatments to be diploids or "$2x$". It is in these natural polyploids that a further duplication of the chromosome complement had been hoped for. Most of them may have reached the limit to which the chromosome complement could be duplicated. Many may not be capable of any further duplication and even if after such a duplication their chromosomal complement is able to function harmoniously, the genetic constitution may make them inferior to the original variety. There is no reason for pessimism just because the possibilities hoped for immediately after the discovery of the effect of colchicine could not be realized in practice. For any planned improvement one has to start from the diploids, wherever available. Polyploidogens would come in handy to convert sterile hybrids from wide crosses into fertile allopolyploids.

The observation that camphor induces gene mutations necessitates the belief that all tetraploids induced by camphor need not be identical in their genic constitution. It is not a mere question of a simple duplication. A doubling of the chromosomes may occur simultaneously in two cells in the

same culture in which camphor had induced mutations in two directions at the same locus or in diverse directions in different loci.

Mitra and Subramaniam (1949) emphasized that polyploidy and endopolyploidy should not be treated as if they are synonymous. A perusal of the cytological pictures observed in fermenting cultures (Figs. 25–32, Subramaniam, 1948 *b*) would reveal that some cells are even 64-ploid. But highly polyploid strains cannot be obtained by isolating such cells from fermenting cultures for the simple reason that they are not viable. Our experience during the past five years leads us to the view that just because high chromosome numbers are observed in fermenting cells, it does not entitle one to believe that viable races with high chromosome numbers could be produced by repeated treatment with polyploidogens. Highly endopolyploid cells occur during the anaerobic phase. Stability and viability is judged by growth under aerobic conditions. The physiology of the cells during the aerobic and anaerobic phases are entirely different and hence polyploidy and endopolyploidy cannot be synonymous.

Effect of Polyploidogens on Endopolyploid Cells.—In the above context, *the effect of polyploidogens on mitotically dividing cells have to be clearly differentiated from those on endopolyploid cells.* The so-called *C-tumour reaction* may be the result of an acceleration of the rate of doubling of the chromosomes in *endopolyploid* nuclei of differentiating tissues. It appears "that growth substances tend to enlarge cells and that chromosome doubling or redoubling follows. The final effect is therefore similar to that of colchicine, but the mechanism is entirely different" (Krythe and Wellensieck, 1942, p. 39; Levan, 1939). It would be desirable to keep in mind the significant fact *that to obtain stable polyploids, the cells in which chromosome duplications had taken place should be viable.* Endopolyploid cells are non-viable and a doubling of the chromosome complement as a result of exposure to colchicine or camphor as suggested by Levan and Sandwall (1943) and Levan (1947) cannot produce any improvement. This may explain their inability to isolate stable polyploid races.

Levan and Sandwall (1943) and Levan (1947) studied the effects of camphor on fermenting cells. Much emphasis has been placed on the so-called appearance of *camphor cells* and *camphor colonies.* Their characteristics are stated to be the non-separation of buds from mother cells with the resultant formation of clusters of cells which cannot be separated even by vigorous shaking. We have repeatedly seen such forms in old cultures of our control preceding autolysis and innumerable records of similar experiences have been reported earlier (Levan, 1947, p. 457). It is these "invo-

lution forms" that have been given undue importance and been made the basis for extensive investigations. It appears highly questionable whether this is necessary or desirable. Even ageing cultures contain a small percentage of mitotically dividing cells and we do not know whether the "involution forms" arise only from the highly endopolyploid cells or from the mitotically dividing cells as well. It appears that the so-called camphor colonies observed by Levan and Sandwall (1943) are of two types. They record: "We made several one cell cultures of such giant cells, and we found that they were usually non-viable. But in those cases where they could be brought into fermentation they had always reverted to the normal size and appearance. The same was the case with those plate cultures which were made from the different treated materials, including five plates of the camphor culture with more than 90% camphor forms. In no case did we obtain any giant colonies with deviating macroscopical appearance. The microscopical examination of some 100 colonies showed the cells to be of normal size and shape" (p. 176). The experience of Skovsted appears to be similar. The germination of the cells of abnormal appearance isolated by a micro-manipulator is stated to be very poor. Thus, Levan and Sandwall (1943), Levan (1947) and Skovsted (1948) seem to have included under their so-called camphor forms two entirely different types of abnormal cells. A clear distinction is necessary between viable and non-viable giant cells. The necessity for differentiating the effect of camphor on endopolyploid fermenting cells and on those capable of normal aerobic proliferation by mitosis is therefore obvious. Just as mere inhibition of cell division by a chemical does not necessarily imply that stable tetraploids would result, mere appearance of the so-called camphor forms does not imply that stable giant forms could be isolated and purified.

Gene Mutations Induced by Camphor.—The experiments on the effect of camphor on fermenting cultures were planned mainly to discover whether a short treatment would induce a doubling of the chromosome complement. It has to be remembered that Levan and Sandwall (1943) and Skovsted (1948) treated fermenting cultures only for short periods. The evidences presented above indicate that they could not have induced a duplication of the chromosome complement even if their starting culture was a diploid. The duration of treatment was very short. Levan and Sandwall judged the changes occurring on treatment by estimating the increase in cell number after 20 and 48 hours. Skovsted (1948) on the other hand, evaluated the changes on the basis of giant colony characteristics. The duration of treatment in only one of his series was extended to 48 hours. The photographs given in this paper give ample evidence that at least in our control

2-chromosome strain, tetraploid sectors appear only in cultures which have been growing in the presence of camphor for about 7 days. This fact is clearly indicated by photos 16 and 23. While a good crop of yeast was obtained 24 hours after the introduction of a loop of cells into the wort incorporated with a drop of camphor, it was only on the 6th day that a fairly good yield was observed in the tube containing two drops of camphor. In correlation with this delayed growth is the fact that the former showed tetraploid sectors in colonies inoculated on the 7th day, while the latter took 13 days to do so.

The surprising fact which emerged was that on treatment with camphor, there is a change in the population of the gene mutants. Photos 1, 6, 7, 8, 9, 14 and 15 give an idea of the normal changes in the population in a growing agar slant. A perusal of the photographs would disclose that the changes in cultures growing in the presence of camphor are not parallel. The question naturally arose whether media containing camphor form selective environments. Photos 2, 5, 11, 12, 14, 16 and 17 indicate that no mutant finds a selective environment, since all the colonies are sectored. The changes produced in the two series of experiments are also not similar. While photos 2 and 5 in the first series and photos 11 and 16 in the second would lead one to suspect that the smoother types are favoured, photos 21 and 23 dispel any such suspicion. Photos 11, 12, 13, 16 and 17 exemplify the changes in the population during the progress of treatment. The valid conclusion that could be drawn is that camphor accelerates the rate of mutation. Photos 6, 7, 8 and 9 of the control inoculated on successive days offer an idea of the normal mutability observed during the period when the control is kept growing on an agar slant. In the above photographs the *Smooth II* sectors are inconspicuous. On the other hand, 24 hours after the commencement of the exposure to camphor there is a predominance of the *Smooth II* cells. Can we conclude that the presence of camphor has accelerated the rate of mutation even during the short exposure of 24 hours ? Comparison of photos 10, 11 and 12 with photos 13, 16 and 17 confirm that there is a persistent accelerated rate of mutation at the locus. After 48 hours of treatment with one drop of camphor solution, the colony is dominated by the *Smooth II* cells (photo 12). But when the treatment is continued for another 24 hours, the colony becomes *Rough II* again. When another 96 hours is allowed to elapse, the *Smooth II* puts in its appearance once more (photo 16). But that is not all. A fairly rare type of sculpturing is also induced (photo 17). These facts emphasize that gene mutations are occurring at an accelerated rate at the locus governing the nature of sculpturing of the colony. That these are not " directed mutations " would be

evident from the appearance of a comparatively rare mutant. It appears to be merely a question of an acceleration. This is exactly what has been claimed for chemical mutagens by Auerbach (1949).

Our experiments on the effect of various chemical and physical agencies on yeasts during the past few years has impelled us to discard the accepted explanation that the doubling of the chromosome complement by polyploidogens is the direct result of an inhibition of cell division. It is known that many chemicals are able to induce the so-called *C-mitosis*, but only a few are capable of producing stable tetraploids. If a doubling of the chromosome complement is a corollary to the induction of the so-called *C-mitosis* it stands to reason that all chemicals which inhibit the anaphase movement should produce stable tetraploids. It appeared to us that an inhibition of the anaphase movement may not after all be an important factor in the production of tetraploids. The stability of the induced tetraploids implies that the duplicated chromosome complement should be capable of functioning harmoniously. Just as gene mutations have been observed to occur spontaneously in our control strain (Subramaniam, Ranganathan and Krishna Murthy, 1948) tetraploidy has also been recorded (Prema Bai and Subramaniam, 1947). The only difference has been that the rate of incidence of autotetraploidy is exceedingly low. Photo 8 in the present paper illustrates a tetraploid sector in the control colony (*cf.* photos 4 and 5, Subramaniam and Krishna Murthy, 1949). Levan and Sandwall (1943) remark that camphor is a C-mitotic substance. The sporadic occurrence of autotetraploidy indicates that a tetraploid complement of chromosomes is able to function harmoniously. If mere inhibition of the anaphase movement is the major factor in the induction of polyploidy, then, camphor which is claimed to be a C-mitotic substance should be capable of causing quickly a doubling of the chromosome complement. In wort containing two drops of camphor a fairly good crop appeared only on the 6th day. It is legitimate to consider that at this concentration, camphor had inhibited cell division for a considerable length of time since only few cells were inoculated at the start of the experiment. But the colonies (photos 18 and 19) which developed after this long inhibition of growth showed no tetraploid sectors. Our experience has been that the effect of polyploidogens is not an all or nothing reaction. Dermen (1940) believes that induction of polyploidy could be expected only when cell division is active. The yeast cells inoculated into the medium containing camphor were dividing actively. Except for the presence of camphor, the cultural conditions at the start of the experiment were ideal for aerobic growth. Camphor is considered to be a C-mitotic substance and hence if induction

of C-mitosis is the major factor in producing stable tetraploids, the majority of the cells in the above camphor culture should have become tetraplcids.

It is emphasized that the duration of treatment " is dependent on the time required for the cycle of cell division in the particular tissue" (Dermen, 1940). Under optimal conditions our control strain divides once in every hour. Therefore, treatment for as short a period as one hour should theoretically be sufficient to produce a C-mitotis. When treatment for 24 hours does not produce even a small number of tetraploids, it is logical to conclude that the so-called C-mitosis which has attracted considerable attention (Levan and Ostergren, 1943; Ostergren, 1944; Levan, 1944) may be unrelated to the induction of polyploidy. Evidence for such a conclusion is found in the published literature. Ostergren (1944) significantly remarks that " the demonstration of a colchicine activity in all these substances does not at all mean that they can all be used for polyploidization purposes " (p. 440). In animals, colchicine usually produces a C-mitosis but only rarely does such an arrest lead to the formation of tetraploid cells. " Significantly, while in animal cells further metamorphosis of chromosomes was stopped and cellular degeneration ensued, in plants the metamorphosis of chromosomes appeared not to be affected; but sister chromosomes being left together in the affected cells, chromosome number was doubled. Even though doubling of the chromosome number has been induced in animal cells, as yet the cells have not survived very long and have not developed either into a polyploid individual or into polyploid tissue " (Dermen, 1940). We find thus that many substances which arrest the anaphase movement do not produce stable polyploids and that colchicine which is capable of inducing polyploidy in plants is unable to do so in animals in spite of its capacity to arrest mitosis. As far back as 1910, Kemp reported that the tetraploid cells produced by treatment with a dilute solution of chloral hydrate show abnormal mitoses and multipolar spindles. Production of stable tetraploids does not therefore depend on an induction of C-mitosis or on the temporary formation of a tetraploid nucleus. The main factor is the ability of the doubled complement of chromosomes to function harmoniously. In this connection, a very suggestive statement of Dobzhansky (1947) is of considerable interest. He remarks that " the doubling of the chromosome complement produces a change in the norm of reaction of the organism and its effect is in this respect analogous to that of a gene mutation " (p. 229). The difference between polyploidogens and those producing C-mitotis is that the former produce a " mutation " making the tetraploid cells viable. Narcosis may result in an arrest of mitosis and this may even be succeeded by the formation of polyploid nuclei. Lacking the

" mutation " which stabilizes the condition, these temporarily polyploid nuclei when they divide give rise to the common aberrations observed even in colchicine treated material. When tetraploidy could be induced by colchicine it would at first sight appear anomalous that some polyploid cells should show multipolar mitoses. *The only possible conclusion is that stable tetraploids arise only when the doubling of the chromosome complement is preceded by a stabilizing gene mutation.* Acceptance of the above suggestion would offer a rational explanation for the mitotic aberrations commonly observed after colchicine treatment. It would be patent that only those nuclei in which the gene mutation has stabilized the condition can multiply normally and give rise to polyploid tissue. In the other temporarily polyploid nuclei in which the stabilizing gene mutation had not occurred, mitotic aberrations should be the normal corollary.

These theoretical possibilities suggested themselves to us even at the time the first tetraploid yeast was produced in this laboratory after treatment of our control two chromosome strain with acenaphthene (Subramaniam, 1945; 1947). It was suspected that if induction of tetraploidy should be preceded by gene mutations, polyploidogens should be capable of producing other gene mutations as well. To offer proof for the above theoretical possibilities, a knowledge of the spontaneous changes that occur in the control culture as well as those that could be induced by diverse agencies, is an essential pre-requisite. A perusal of the extensive literature on the effect of polyploidogens on higher plants would indicate that plants may not form an ideal material for any detection of gene mutations that may be induced by polyploidogens. The obvious choice is of course a suitable micro-organism. But micro-organisms present other problems. Their cytology is little known. A co-ordinated plan of investigations on the Cytogenetics of Yeasts has enabled us to distinguish a duplication of the chromosomal complement from chromosomal translocations and both of the above categories from gene mutations (Subramaniam, Ranganathan and Krishna Murthy, 1948; Subramaniam, 1950). Sporadic but spontaneous gene mutations have been reproduced by exposure to ultra-violet irradiation (Subramaniam and Krishna Murthy, 1948; Subramaniam and Ranganathan, 1949). When tetraploidy occurs spontaneously in a manner comparable with gene mutations under normal environmental conditions, the probability that a specific gene mutation should precede a doubling of the chromosome complement gets added confirmation. The fact that camphor which induces polyploidy also accelerates the rate of mutation at the locus governing the nature of sculpturing of the giant colony appeared to us to be a remarkable confirmation of the above theoretical possibilities

(Subramaniam and Sreepathi Rao, 1950). This finds added emphasis in the fact that while the control inoculated on 24th of August 1949 (photo 8) showed a tetraploid sector, the colonies of cultures treated with camphor and grown simultaneously did not show any such sectors (photos 10 and 11). While a gene mutation is very common, spontaneous tetraploidy is a relatively rare phenomenon in our control strain. It naturally follows that the mutation rate at the locus for tetraploidy should be very low. If camphor accelerated the rate of mutation at the different loci to the same degree, even then, incidence of tetraploidy should be much less than mutations at the locus governing the nature of sculpturing of the colony. This is exactly what the results with camphor treatment demonstrate. While gene mutants appear after treatment for 24 hours (photos 2, 3, 10 and 11), tetraploid sectors were observed only after exposure for 6 days. Added confirmation is offered by the behaviour of the cells inoculated into wort containing two drops of camphor. A crop was observed only on the 6th day. But such long inhibition of growth did not induce polyploidy. Tetraploids appeared only on the 13th day, *viz.*, 6 days after the appearance of an yeast crop in the tube. *Inhibition of cell division and induction of tetraploidy appear thus to be unrelated phenomena.* It is a common observation that after colchicine treatment some aberrant diploids are produced. We have observed the same phenomenon in yeasts also. After exposure of an active aerobic culture of our control to colchicine for 40 days we obtained in addition to the tetraploids, a diploid also (Ranganathan and Subramaniam, 1950). Such a prolonged treatment did not induce the gene mutation necessary to convert all the cells of the culture into stable tetraploids.

There is considerable justification for the belief that a gene mutation precedes any induction of tetraploidy. The autotetraploid BY 3, isolated by us in 1945 after treatment of the two chromosome control with acenaphthene (Subramaniam, 1945; 1947) is highly stable. It forms spores and such spores should have balanced diploid chromosome complements. As such the spores should be capable of direct germination with the resultant formation of diploid vegetative cells. During the past four years, however, no diploid sectors have appeared in any of our tetraploid colonies. It is a common observation that some asci contain only a single spore. The natural tendency, in view of its possession of a balanced diploid complement of chromosomes, should be to germinate directly and give rise to diploid cells. When that does not happen it is reasonable to infer that the spores have a tendency to fuse before germination. This can happen only when there is a mutation preceding the induction of tetraploidy making the spores complementary. It is this change probably, that is exhibited

by the mating types of Lindegren (1945; Subramaniam, 1950). The description of a gene for diploidization described by Winge and Roberts (1948) is reminiscent of such a condition. An extended discussion has already been given elsewhere (Subramaniam, 1950).

On the above basis it is easy to explain why all the chemicals which induce the so-called C-mitosis do not produce stable tetraploids. From the evidence in published literature it appears that only a few are capable of inducing a mutation at the locus enabling the duplicated complement of chromosomes to function harmoniously. The fact that colchicine and acenaphthene induce the gene mutation for production of stable tetraploids implies that these chemicals should be capable of accelerating the mutations at other loci as well.

Navashin (1938) observed the occurrence of haploid branches in *Crepis capillaris* after treatment with acenaphthene. Levan (1945) seems to have obtained a haploid sugar beet after colchicine treatment. If one assumes the probability that a series of alleles at a particular locus governed the harmonious working of the diploid and higher polyploid chromosome numbers, then the results obtained by Navashin and Levan do not appear to be surprising at all. Even in yeasts the results obtained by Skovsted (1948) suggest that a reversion to the haploid condition may have occurred as a result of treatment with camphor. On the basis of the results obtained in this laboratory it was suspected that the alcohol may have been responsible for the probable occurrence of "haploidy" in Skovsted's experiments (Duraiswami and Subramaniam, 1950). A slightly different explanation is now possible.

It was emphasized in the historical resumé that Skovsted's identification of his control strain as a "haploid" was of a questionable nature. On treatment, he observed the appearance of cells which developed into dwarf colonies. The cells from a crenated dwarf colony illustrated by him in his Fig. 1 reminded us of the large cells observed in this laboratory in the unstable haploid obtained from our two chromosome control on continued culturing in the presence of a high percentage of alcohol (Duraiswami and Subramaniam, 1950). The colony of our unstable haploid was a dwarf but the cells were bigger in size than those from the giant colony of the control. The above observation that the haploid may be bigger in size than the control is confirmed by Satava's observations (Winge and Laustsen, p. 107). In his classification of the "reduced" forms, the type III is stated to be unable to regulate the shape and size of the cells. Naturally these were irregular, longish and often amœboid. A comparison of Skovsted's figures

with Satava's description suggests that his dwarf colonies may be assigned to the type III of reduced forms observed by Satava. Actually, therefore, instead of producing a chromosomal duplication, camphor may have induced haploidy in Skovsted's experiments. If it is permissible to extend the conclusion drawn from the observations recorded in this paper to those of Skovsted, it appears that camphor had accelerated the rate of mutation in his strain also and that the sectors observed by him may be the result of gene mutations.

When mutational rates of different loci differ as shown by the results presented in this paper, it may not be surprising if the locus for polyploidy shows different mutational rates in different organisms. Levan (1939) observed that tetraploidy and even rarely octoploidy could be induced in *Petunia* by a single application of colchicine. He continues: "This behaviour of *Petunia* is especially striking in comparison with other experimental plants, which are often very difficult to change into tetraploidy. *Petunia's* antipode in this respect is *Beta* in which I carried out similar investigations parallely. In *Beta* the young growing point reverts stubbornly back into the diploid status, even after 10 applications of the same kind of colchicine agar as used in *Petunia*" (Levan, 1939, p. 125). Some diploid *Petunia* produce spontaneously tetraploid cells and sectors. But they are said to remain diploid. The differences in the behaviour of *Petunia* and *Beta* cannot, therefore, be merely due to differences in the relative growth rate and viability of the diploid and tetraploid cells in the two genera. It is quite probable that they may differ in the rate of mutation at the locus for polyploidy. In animals polyploidy is rare, and it is quite likely that the absence of a locus may be responsible for the failure to induce polyploidy by agencies commonly producing such changes in plants. It has to be remembered that colchicine does induce a chromosome doubling, only, the absence of a gene prevents the harmonious working of the polyploid complement of chromosomes.

Summary

1. The effect of camphor on fermenting yeast cultures is described. The necessity for a clear differentiation of the effect of camphor on aerobically growing cultures from that on fermenting cells is emphasized.

2. Tubes containing one, two, three, four and five drops of a known concentration of an alcoholic solution of camphor incorporated in 5 ml. of wort were inoculated with a few cells from an aerobically growing culture of the two chromosome control strain. Giant colony inoculations were

carried out 24, 48, 72 and 168 hours after the commencement of the experiment without any fresh addition of camphor.

3. The first series of giant colonies indicated that camphor had induced gene mutations in several directions. In the second series the changes in the population of the control growing on an agar slant was investigated side by side with the study of the mutations induced by camphor. The changes in the population of the gene mutants in the culture of the control and that growing in the presence of one or two drops of camphor solution are not parallel.

4. Camphor accelerates the mutation rate at the locus governing the nature of sculpturing of the colony. It is suggested that stable tetraploids arise only when the doubling of the chromosome complement is preceded by a specific stabilizing gene mutation. Since camphor induces gene mutations, all tetraploids induced by camphor need not have identical genic constitution.

5. If one assumes the probability that a series of alleles at a particular locus govern the harmonious working of haploid, diploid and polyploid chromosome numbers, treatment with polyploidogens instead of doubling the chromosome complement may even lead to production of forms with reduced chromosome numbers.

ACKNOWLEDGMENT

We are very grateful to the Council of Scientific and Industrial Research for generous financial assistance.

REFERENCES

1. Auerbach, C. .. "Chemical Mutagenesis," *Biol. Rev.*, 1949, **24**, 355–91.
2. ———— .. "Chemical Induction of Mutations," *Proc. 8th Internat. Cong. Gen.*, 1949 *b*, *Hereditas* Suppl.
3. Badian, J. .. "Sur la Cytologie des Levures," *Bull. Internat. Acad. Polonaise. Sci. et Lettr. Cl. Sci. Ser. B. Sci. Nat.*, 1937, **1**, 61–87.
4. Bauch, R. .. "Experimentelle Mutationsauslosung bei Hefe und anderen Pilzen durch Behandlung mit Kamfer, Acenaphthene und Colchicin," *Naturwiss*, 1941, **29**, 503–04.
5. Dermen, H. .. "Colchicine Polyploidy and Technique," *Bot. Rev.*, 1940, **6**, 599–635.
6. Dobzhansky, Th. .. "*Genetics and the Origin of Species*, 1947. Columbia Univ. Press.
7. Duraiswami, S. and M. K. Subramaniam .. "Reversal of Some Chromosomal Mutations in Yeasts," .. *Cellule*, 1950, **53**, 215–56.
8. Guilliermond, A. .. *The Yeasts*, 1920. John Wiley & Sons, New York.
9. Kater, J. McA. .. "Amitosis," *Bot. Rev.*, 1940, **6**, 164–80.

10. Kemp, H. P. .. "On the Question of the Occurrence of Heterotypical Reduction in Somatic Cells," *Ann. Bot.*, 1910, **24**, 775–803.
11. Krishna Murthy, S. N. and M. K. Subramaniam "Further Observations on Reverse Mutations in Yeasts," *J. Ind., Inst. Sci.*, 1950, **32 A**, 1–28.
12. Krythe, J. M. and S. J. Wellensieck .. "Five Years of Colchicine Research," *Bibl. Gen.*, 1942, **14**, 1–132.
13. Levan, A. .., "Cytological phenomena connected with root swelling caused by growth substances," *Hereditas*, 1939, **25**, 87–96.
14. ———— .. "A Haploid Sugar Beet After Colchicine Treatment," *Ibid.*, 1945, **31**, 399–410.
15. ———— .. "Studies on the Camphor Reaction of Yeast," *Ibid.*, 1947, **33**, 457–514.
16. Levan, A. and G. Ostergren .. "The Mechanism of C-Mitotic Action," *Ibid.*, 1943, **29**, 381–443.
17. ——— and C. G. Sandwall "Quantitative Investigations on the Reactions of Yeast to Biologically active substances," *Ibid.*, 1943, **29**, 164–78.
18. Lindegren, C. C. .. "Mendelian and Cytoplasmic Inheritance in Yeasts," *Ann. Mo. Bot. Gdn.*, 1945, **32**, 107–23.
19. Mallya, Prema Bai, and M. K. Subramaniam .. "Genic Differences and Rate of Growth in Yeasts," *Nature*, 1949, **163**, 251–52.
20. Mitra, K. K. .. "Autotetraploidy and Attenuating Power in Yeasts," *Curr. Sci.*, 1948, **17**, 55.
21. ——— and M. K. Subramaniam .. "Some Observations on the Effect of Chrysene on Yeasts," *Cellule*, 1949, **53**, 7–12.
22. Navashin, M. .. "Influence of Acenaphthene on the Division of Cells and Nuclei," *C. R. Dokl. Acad. Sci. U.R.S.S.*, 1938, **19**, 193–96.
23. Ostergren, G. .. "Colchicine Mitosis, Chromosome Contraction, Narcosis and Protein Chain Folding," *Hereditas*, 1944, **30**, 429–67.
24. Prema Bai, M. and M. K. Subramaniam .. "Rate of Growth of Diploid and Tetraploid Yeasts," *Curr. Sci.*, 1947, **16**, 380–81.
25. Ranganathan, B. and M. K. Subramaniam .. "Centrosomes in Yeasts," *Sci. and Cult.*, 1947, **12**, 478–81.
26. ———— .. "Studies on the Cytology of Yeasts. V. Normal and Abnormal Mitoses in a Distillery Yeast," *Proc. Nat. Inst. Sci. India*, 1948, **14**, 389–405.
27. ———— .. "Studies on the Mutagenic Action of Physical and Chemical Agencies on Yeasts. I. Induction of Polyploidy by Diverse Agencies," *J. Ind. Inst. Sci*, 1950, **32 A**, 51–72.
28. Sinoto, Y. and A. Yuasa .. "Karyological Studies in *Saccharomyces cerevisiæ*," *Cytologia*, 1941, **11**, 464–72.
29. Skovsted, A. .. "Induced Camphor Mutations in Yeasts," *C. R. Lab. Carlsberg. Ser. Physiol.*, 1948, **24**, 249–261.
30. Sonneborn, T. M. .. "Recent Advances in the Genetics of Paramecium and Euplotes," *Adv. Gen.*, 1947, **1**, 263–358.
31. Subramaniam, M. K. .. "Induction of Polyploidy in *Saccharomyces cerevisiæ*," *Curr. Sci.*, 1945, **14**, 234.

32. Subramaniam, M. K. .. "Studies on the Cytology of Yeasts. I. Mitosis in *Saccharomyces cerevisiæ*," *Proc. Nat. Inst. Sci. India*, 1946, **12**, 143–49.

33. ———— .. "Studies on the Cytology of Yeasts. II. Induction of Polyploidy and Heterochromatin," *Ibid.*, 1947, **13**, 129–39.

34. ———— .. "Endopolyploidy in Yeasts," *Curr. Sci.*, 1947 *b*, **16**, 157–58.

35. ———— .. "Is the Macronucleus of Ciliates Endopolyploid ?", *Ibid.*, 1947 *c*, **16**, 228–29.

36. ———— .. "Studies on the Cytology of Yeasts. III. Technique of Handling Yeasts for Cytological Investigations," *Proc. Nat. Inst. Sci. India*, 1948 *a*, **14**, 315–23.

37. ———— .. "Studies on the Cytology of Yeasts. IV. Endopolyploidy in Yeasts," *Ibid.*, 1948 *b*, **14**, 325–34.

38. ———— .. "The Problem of Haploidy in Yeasts," *J. Ind. Inst. Sci.*, 1950 *a*, **32 A**, 29–40.

39. ———— .. "Haploidy and the Species Concept in Yeasts," *Ibid.*, 1950 *b*, **32 A**, 41–50.

40. ———— .. "A Critical Evaluation of the Question of Cytoplasmic Inheritance in Yeasts," *Ibid.*, 1950 *c* **32 A**, 73–90.

41. ———— .. "Alleles and Their Time of Expression in Yeasts," *Proc. Nat. Inst. Sci.*, 1951, **17**, 367–371.

42. Subramaniam, M. K. and S. N. Krishna Murthy "A Reverse Mutation in Yeast Induced by Ultra Violet Irradiation," *Curr. Sci.*, 1948, **17**, 92.

43. ———— .. "Effect of Acenaphthene on Yeast Strains of Different Genic and Chromosomal Constitutions," *Proc. Ind. Acad. Sci.*, 1949, **30 B**, 185–94.

44. Subramaniam, M. K. and B. Ranganathan "Peculiar Cytological Behaviour of a Distillery Yeast," *Nature*, 1946, **157**, 50.

45. ———— .. "Chromosome Constitution and Characteristics of Giant Colonies in Yeasts," *Proc. Nat. Inst. Sci., India*, 1948, **14**, 279–83.

46. ———— .. "A Gene Determining Growth Rate in Yeast," *J. Sci. and Ind. Res.*, 1949, **8 B**, 5–9.

47. ——— and S. K. Sreepathi Rao .. "Gene Mutations Induced by Camphor in Yeast," *Research*, 1950, **3**, 49–50.

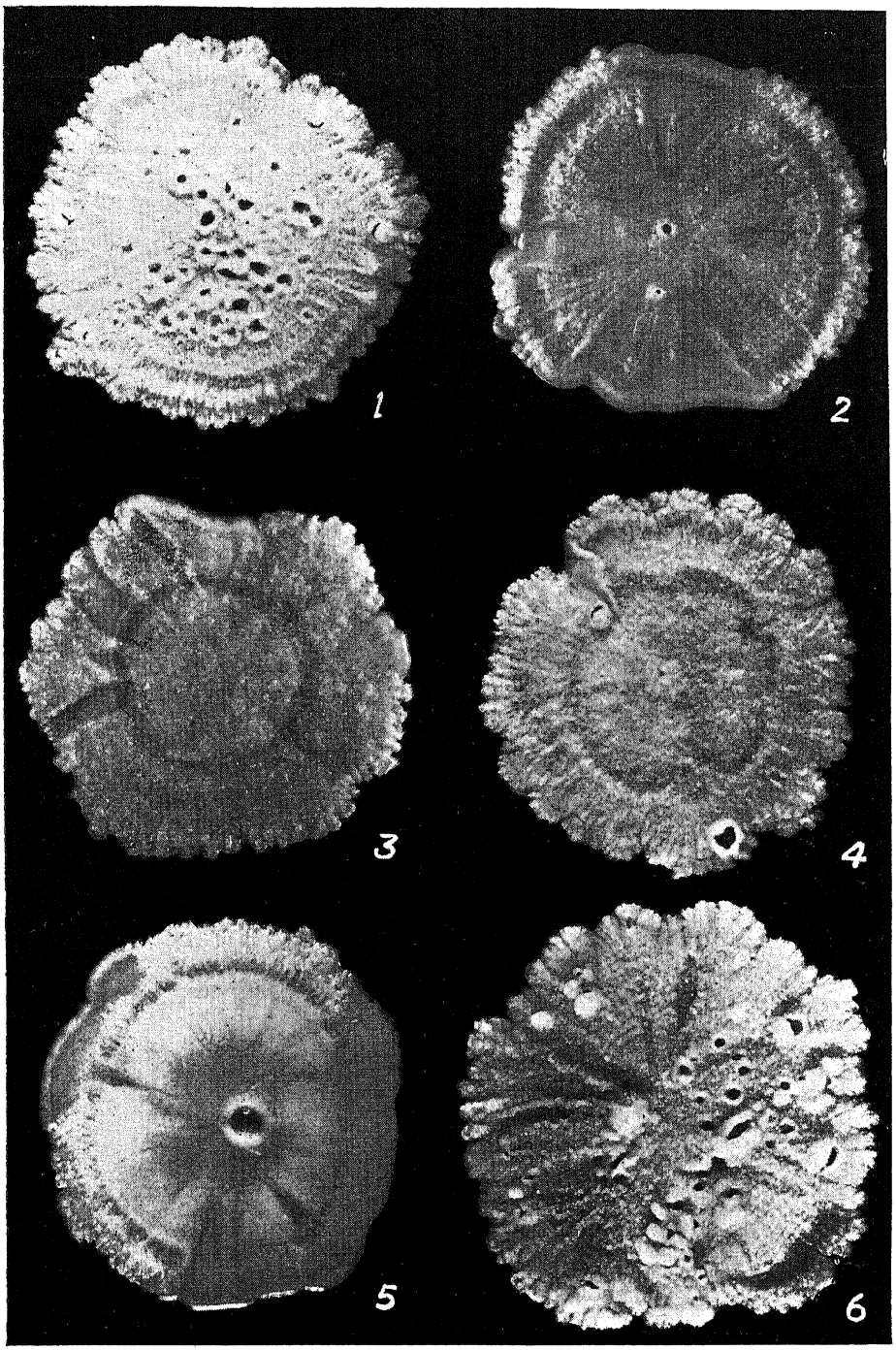
48. ———, B. Ranganathan and S. N. Krishna Murthy "Reverse Mutations in Yeasts," *Cellule*, 1948, **52**, 39–60.

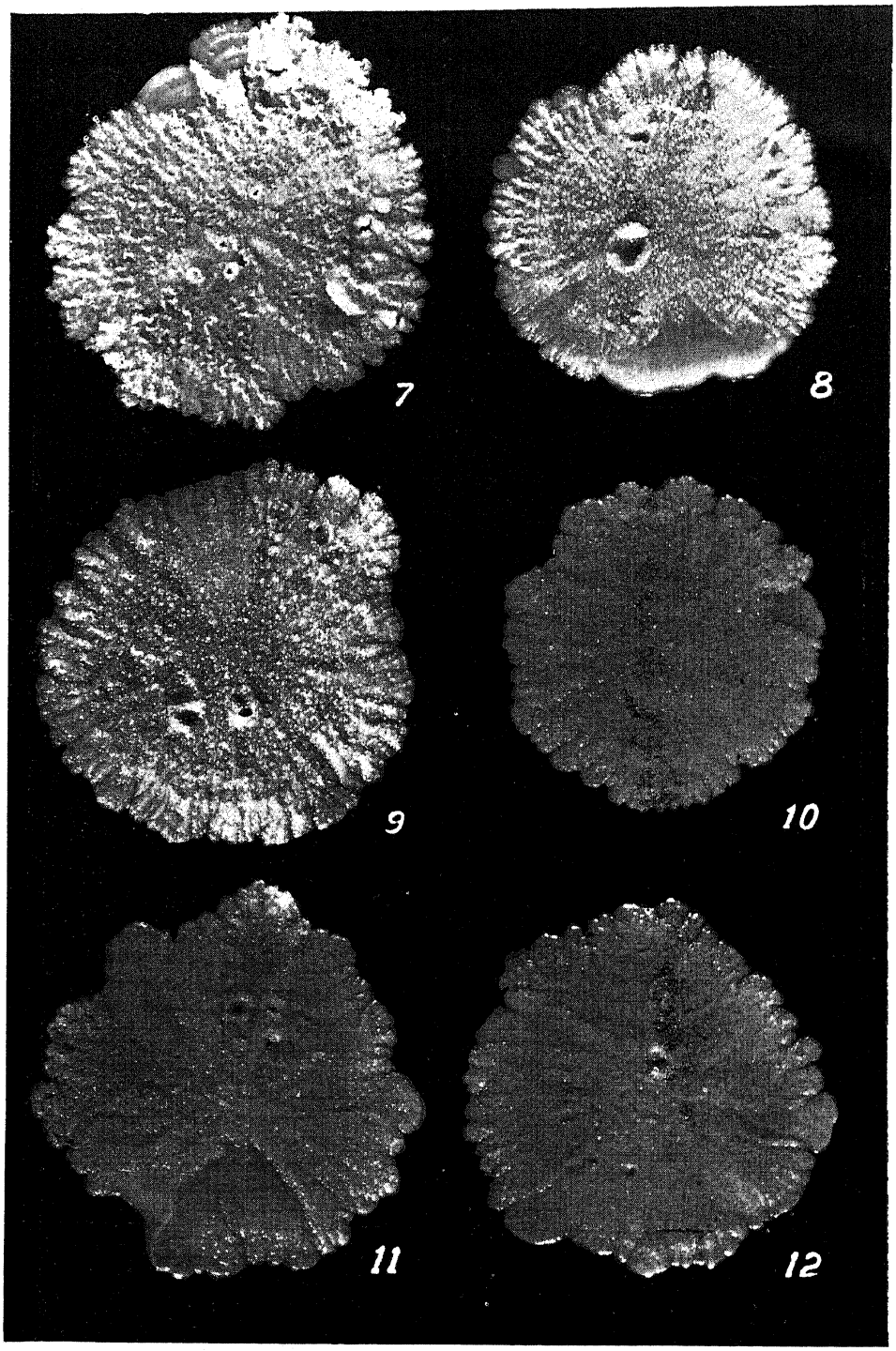
49. Winge, O. .. "On Haplophase and Diplophase in Some Saccharomycetes," *C. R. Lab. Carlsberg. Ser. Physiol.*,1935, **21**, 77–112.

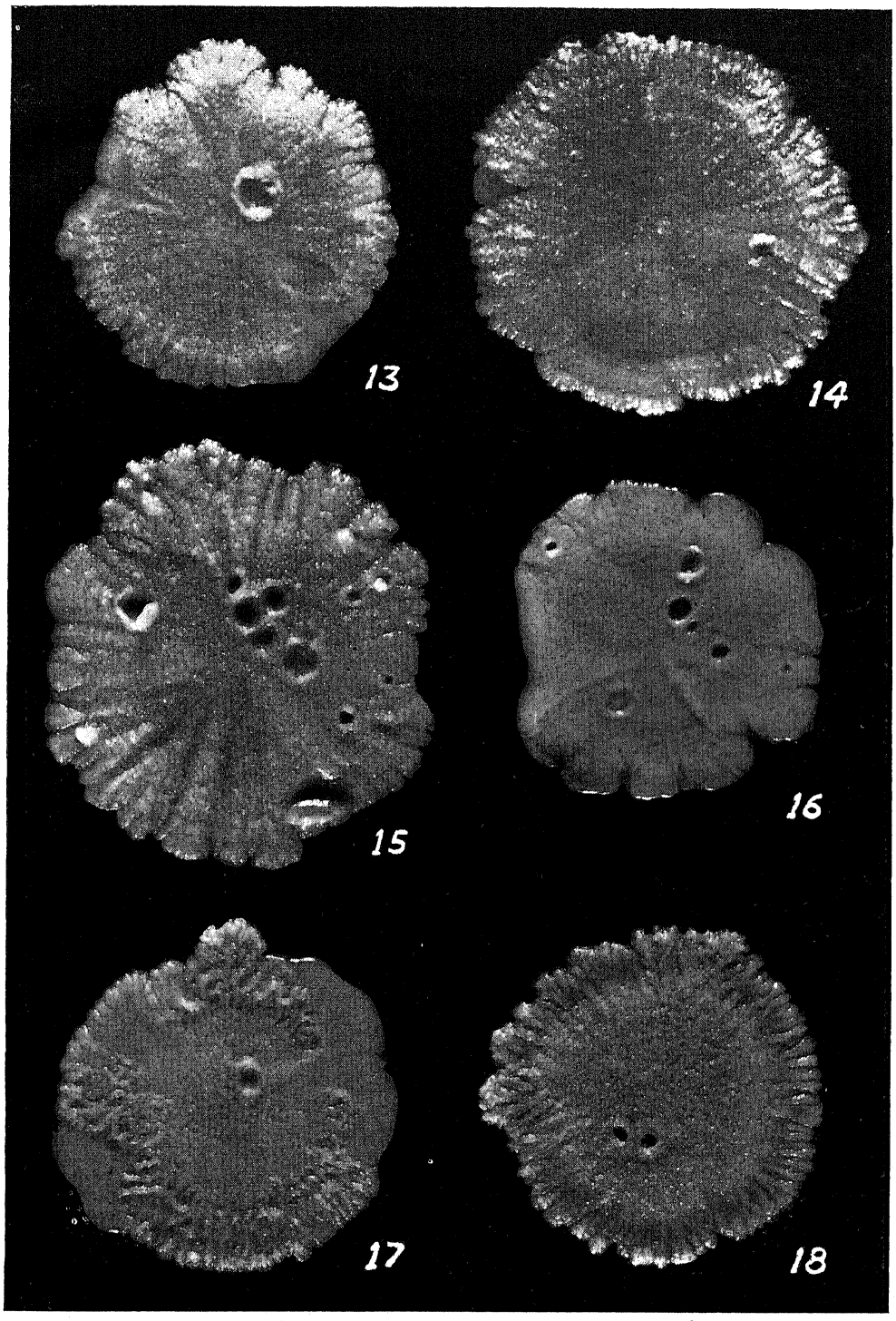
50. ——— and O. Laustsen .. "On Two Types of Spore Germination and on Genetic Segregations in *Saccharomycetes* demonstrated through single spore cultures," *Ibid.*, 1937, **22**, 99–120.

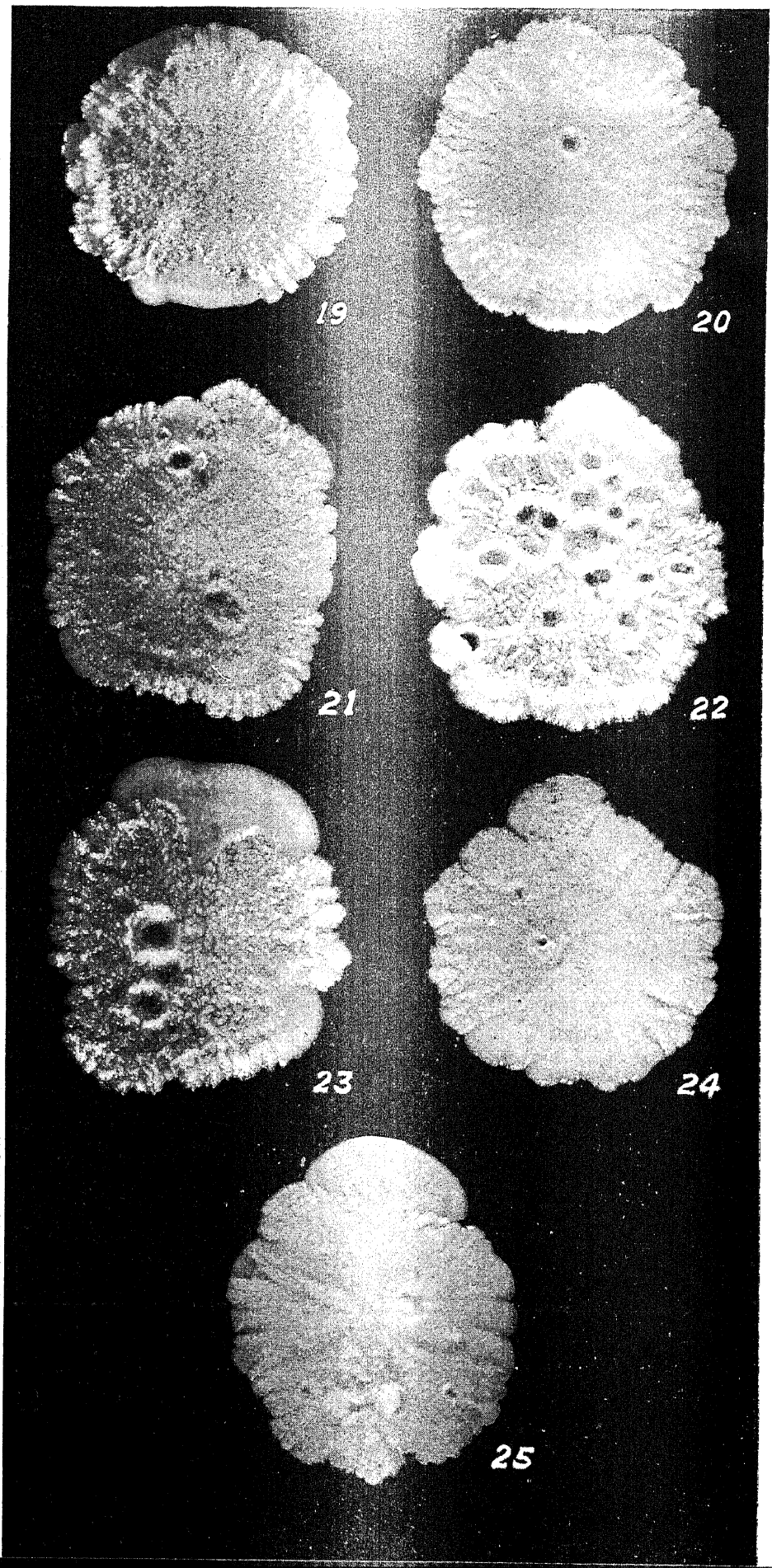
51. ———— .. "On the Cytoplasmic Effect of Inbreeding in Homozygous Yeasts," *Ibid.*, **23**, 17–39.

52. Winge, O. and C. Roberts .. "Inheritance of Enzymatic Characters in Yeast and the Phenomenon of Long Term Adaptation," *Ibid.*, 1948, **24**, 263–315.


1
2
3
4
5
6


7
8
9
10
11
12


13
14
15
16
17
18


19
20
21
22
23
24
25

Description of Photographs

Photo 1. BY 1 (12-10-45), 3·2 cm., 16 days growth, photographed on 22-8-49.

Photo 2. BY 1 grown in wort containing one drop of camphor solution for 24 hours, 2·9 cm., 15 days' growth, 27-8-49.

Photo 3. BY 1 grown in wort containing one drop of camphor solution for 24 hours, 3·3 cm., 15 days' growth, 26-8-49.

Photo 4. BY 1 grown in wort containing one drop of camphor solution for 48 hours, 3·4 cm., 15 days' growth, 26-8-49.

Photo 5. BY 1 grown in wort containing one drop of camphor solution for 48 hours, 2·4 cm., 16 days' growth, 27-8-49.

Photo 6. BY 1 (12-10-45), 3·2 cm., 16 days' growth, 8-9-49.

Photo 7. BY 1 (12-10-45), 3·5 cm., 16 days' growth, 8-9-49.

Photo 8. BY 1 (12-10-45), 3·0 cm., 15 days' growth, 8-9-49.

Photo 9. BY 1 (12-10-45), 3-4 cm., 15 days' growth, 8-9-49.

Photo 10. BY 1 (No. 1) grown in wort containing one drop of camphor solution for 24 hours, 3·3 cm., 15 days' growth, 8-9-49.

Photo 11. BY 1 (No. 1) grown in wort containing one drop of camphor solution for 24 hours, 4·2 cm., 15 days' growth, 8-9-49.

Photo 12. BY 1 (No. 1), grown in wort containing one drop of camphor solution for 48 hours, 3·7 cm., 19 days' growth, 13-9-49.

Photo 13. BY 1 (No. 1), grown in wort containing one drop of camphor solution for 72 hours, 3·0 cm., 18 days' growth, 13-9-49.

Photo 14. BY 1 (12-10-45), 3·4 cm., 18 days' growth, 13-9-49.

Photo 15. BY 1 (12-10-45), 3·1 cm., 14 days' growth, 13-9-49.

Photo 16. BY 1 (No. 1) grown in wort containing one drop of camphor solution for 7 days, 3·4 cm., 14 days' growth, 13-9-49.

Photo 17. BY 1 (No. 1) grown in wort containing one drop of camphor solution for 7 days, 2·5 cm., 14 days' growth, 13-9-49.

Photo 18. BY 1 (No. 2), grown in wort containing two drops of camphor solution for 6 days, 2·8 cm., 17 days' growth, 14-9-49.

Photo 19. BY 1 (No. 2), grown in wort containing two drops of camphor solution for 6 days, 3·2 cm., 17 days' growth, 14-9-49.

Photo 20. BY 1 (No. 2), grown in wort containing two drops of camphor solution for 8 days, 3·3 cm., 15 days' growth, 14-9-49.

Photo 21. BY 1 (No. 2), grown in wort containing two drops of camphor solution for 8 days, 3-9 cm., 15 days' growth, 14-9-49.

Photo 22. BY 1 (No. 2) grown in wort containing two drops of camphor solution for 13 days, 2·7 cm., 11 days' growth, 15-9-49.

Photo 23. BY 1 (No. 2) grown in wort containing two drops of camphor solution for 13 days, 2-9 cm., 11 days' growth, 15-9-49.

Photo 24. BY 1 (12-10-45), 3·2 cm., 11 days' growth, 15-9-49.

Photo 25. BY 1 (12-10-45), 3·4 cm., 11 days' growth, 15-9-49.

All the giant colonies were grown on Barley Malt Agar.