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

V. Effect of Ultraviolet Irradiation on a Diploid Brewery Yeast

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Received August 13, 1951

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Introduction

Reversal of gene mutations (Subramaniam and Krishna Murthy, 1948) as well as an induction of tetraploidy (Ranganathan and Subramaniam, 1950) was observed in this laboratory when our two chromosome brewery yeast was exposed to ultra-violet irradiation. Recent investigations revealed the mutagenic action of polyploidogens on yeast. It has been shown that camphor (Subramaniam and Sreepathi Rao, 1950; Ranganathan and Subramaniam, 1950), colchicine (Ranganathan and Subramaniam, 1950), acenaphthene (Duraiswami and Subramaniam, unpublished) and chrysene (Mitra and Subramaniam, 1949, 1950) like thermal shocks (Ranganathan, 1950; Subramaniam and Prahlada Rao, 1951) produce not only a doubling of the chromosome number but accelerate also the mutations at the various loci. The action of polyploidogens, therefore, does not consist in a mere duplication of the chromosome number, but involves, primarily, a gene mutation enabling such a doubled complement to function harmoniously. It has been conceived that there should be a locus for polyploidy and that the presence of different alleles at this locus governs the viability of specific chromosome numbers. Indirect evidence in support of the above

contention is afforded by the results from irradiation experiments. Chromosomal variants as well as polyploid and haploid types have arisen as a result of irradiation. Goodspeed (1936) in fact suggests that the alterations in the chromosome numbers may be the result of induced gene mutations. If that is so, tetraploidy in yeast should be sporadic and exposure to ultra-violet light need not always result in an induction of polyploidy. The experiments reported here were planned to check the above possibility.

Another interesting fact appears to be the similarity in the explanations offered for the mutagenic action of chemical and physical agencies. Chrysene which induces tetraploidy accelerates the rate of gene mutations. Ultra-violet irradiation which is said to accelerate gene mutations has been shown to induce tetraploidy in our control strain.

MATERIAL AND METHODS

To facilitate correct interpretation of the data, it was thought desirable to start with a diploid culture pure for a given type of sculpturing of the giant colony. Photo 1 shows the appearance of a colony of the control diploid brewery yeast inoculated on 30–8–1949 and photographed on 13–9–1949 at the end of 14 days' growth. It is pure for the *Rough II* type of sculpturing. A loop of material was taken from the periphery of the above colony and transferred to 5 ml. of barley malt wort in a bacteriological test tube. This constituted the starting material for the present investigation and was labelled BY 1S. After allowing the above liquid culture to grow for 24 hours, loops were transferred to two sterile vitreosil assay tubes each containing 2·5 ml. of wort. Enough number of loops were inoculated into each of the tubes to give a good turbidity, thus providing for a high survival percentage after the irradiation.

The two assay tubes were next placed at a distance of 90 cm. from an ordinary laboratory mercury arc lamp and irradiated for 4 hours. Care was taken to see that uniform suspensions of the culture were exposed by periodic shaking. The small diameter of the tubes ensured against screening effects due to the thickness of the suspension. At the end of the irradiation, the cultures were labelled UV 1 and UV 2, shaken well and left inside a sterile chamber to proliferate. After 24 hours they were again shaken well and inoculations in duplicates were carried out for giant colonies. At the same time, colonies of the control BY 1S were also run from a 24-hour old liquid culture. These are illustrated in Photos 2, 3, 6, 7, 8 and 9.

The cultures UV 1 and UV 2 were given a change of medium immediately after the inoculations for the giant colonies. Six hours later, two loops from each of the well shaken cultures were inoculated into fresh tubes of wort. The material obtained in each case at the end of growth for 16 hours was plated out by a routine procedure followed in our laboratory. To assess whether any variants had appeared in the control, a 16-hour old culture of BY 1S was also plated out at the same time. After the lapse of a week, two colonies of the control and four each from the plates of UV 1 and UV 2 were picked out on the basis of morphological characteristics for detailed investigation. Thus there were two Controls, 1 and 2, and eight cultures derived from the irradiated material, CYG 1–4 from UV 1 and CYG 5–8 from UV 2. The following table gives in detail all the relevant data:—

Strain	Source	Morphological Characteristics
Control 1	Dilution plate of BY 1 S	Circular colony, wavy margin, rough.
Control 2		Circular colony, entire margin, smooth.
CYG 1	Dilution plate of UV 1	Big, circular, rough colony, wavy margin.
CYG 2		Small, heart-shaped, rough colony, wavy margin with a notch at the centre.
CYG 3		Big, microscopically rough but macroscopically smooth colony.
CYG 4		Small, smooth colony.
CYG 5	Dilution plate of UV 2	Smoothish colony, big, circular, entire margin.
CYG 6		Small, smoothish, yellowish colony.
CYG 7		Very small, slightly notched colony.
CYG 8		Big, smoothish colony, entire margin.

After growth for 24 hours in wort, the above strains were transferred to agar slants. A week later, these were tested for their giant colony characteristics by inoculations from 16-hour old wort cultures. The colonies of CYG 1 and 5 got contaminated during the early phases of growth and as such had to be rejected. The others are illustrated in Photos 4, 5, 10, 11, 12, 14, 15 and 16.

To test whether delayed effects due to the irradiation could be observed, the giant colony characteristics of the above strains were checked along with those of the controls after more than a month. These inoculations were performed successively for the various strains and Photos 17, 18, 19, 20, 21, 22, 23, 24, 25 and 26 illustrate the colonies obtained.

OBSERVATIONS

At the start of the experiment, the control had the *Rough II* type of sculpturing (Photo 1; *cf.* Photo 10, Subramaniam, Ranganathan and Krishna Murthy, 1948; Photos 9 and 10, Duraiswami and Subramaniam, 1950) and had no sectors. The mark left by the loop when material was removed from this colony could be seen as a dent in the margin. When grown for 24 hours in wort, the culture had become a mixture. The composite type of sculpturing observed in Photos 2 and 3 is the result of the mutants composing them having an identical growth rate. When they have different growth rates, the colonies are sectored. This curious feature has been repeatedly observed in this laboratory. From dilution plates the types could be isolated separately. Photos 4 and 5 show the two mutants responsible for the mixed nature of the sculpturing in Photos 2 and 3. The colony in Photo 4 is of the characteristic *Smooth III* type (*cf.* Photo 14, Subramaniam, Ranganathan and Krishna Murthy, 1948), while that in Photo 5 could be assigned to the *Rough I* class. The slightly different appearances of Photos 2 and 3 are probably the result of the two mutants not occurring in the same proportion. It is these types which have been exposed to the action of ultra-violet light. Photos 6 and 7 illustrate the giant colonies grown from the first tube labelled UV 1. Both belong to the *Rough* category with a rim (*Rough III*). In Photo 6 there are a few *Smooth* sectors one of which alone starts from the centre. Photo 7 on the other hand is pure for the type. The *Rough III* (Krishna Murthy and Subramaniam, 1950) has been assumed to be of the genic constitution *Rough/Rim*. The cells before irradiation had the genic constitution *Lace/Lace* (*Smooth III*, Photo 4) and *Rough/Smooth* (*Rough I*, Photo 5). After irradiation the genic constitution has changed to *Rough/Rim*. Ultra-violet irradiation should therefore have produced the following alterations:—

Smooth III	Lace	→ mutation →	Rough	*Rough III*
	Lace	→ mutation →	Rim	
Rough I	Smooth	→ mutation →	Rim	*Rough III*
	Rough	——————→	Rough	

The changes in the second irradiated culture, UV 2 are not strictly comparable to the previous one. Photo 8 is of the *Rough II* category, but it also shows a broad *Smooth* sector extending from the centre to the periphery. From its shape it could be presumed that its rate of growth is inferior to the rest of the colony. This *Smooth* sector could have originated either from the *Smooth III* or from the *Rough I* cells present before the irradiation.

$$\textit{Smooth III} \begin{cases} \text{Lace} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Lace} \rightarrow \text{mutation} \rightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

$$\textit{Rough I} \begin{cases} \text{Rough} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Smooth} \longrightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

A single gene mutation would convert the *Rough I* into the *Smooth I* type. The *Smooth I* sector is likely to have originated, therefore, from the *Rough I* cells. Photo 9 on the other hand, resembles the control at the start of the experiment (Photo 3). It also shows a *Smooth I* sector.

Immediately after the giant colony inoculations of the irradiated material, the cultures were plated as described before. The strains isolated from the plates were now tested for their giant colony characteristics. The colony of CYG 1 got contaminated and had to be rejected. CYG 2 developed into a colony as illustrated in Photo 10. Its sculpturing is reminiscent of the *Smooth III* condition, but it does not appear to be pure for the type. The minute granules scattered especially in the outer half of the colony suggest that the Rough cells are persisting. Before plating, the material was of the *Rough III* type (Photos 6 and 7). During proliferation after irradiation, therefore, mutations should have been going on, reversing the changes induced by exposure to ultra-violet. This phenomenon is much more pronounced in the strain CYG 3 (Photo 11) which has reverted to the condition of the control before treatment with the radiation (*cf.* Photo 3). It is quite likely that the action of ultra-violet consists merely in a temporary acceleration. The increased rate of mutability of the locus seems to persist for some time. While in CYG 3 the reversion was to the original condition, CYG 4 confirms the belief that mutation need not always be in the reverse direction. The colonies illustrated in Photos 12 and 13 are perfectly smooth and entirely different from the control and the other strains isolated from the culture UV 1. Immediately after irradiation, the colony was composed of *Rough III* cells. The following changes should therefore have occurred when cultured after exposure:—

$$\textit{Rough III} \begin{cases} \text{Rough} \rightarrow \text{mutation} \rightarrow \text{Smooth} \\ \text{Rim} \rightarrow \text{mutation} \rightarrow \text{Smooth} \end{cases} \textit{Smooth I}$$

The four strains isolated from the second irradiated tube, UV 2, show a different type of change. The giant colony of CYG 5 got contaminated and had to be discarded. CYG 6 gave a characteristic *Rough I* colony which had a broader sculptured outer region. It is pure and shows no sectors. Immediately after irradiation UV 2 was a mixture of *Rough III*, *Rough I*, *Smooth III* and *Smooth I* categories of cells. It is difficult therefore to evaluate the exact changes. CYG 7 (Photo 15) gave a *Rough I* colony in which the granulations obscured the radial and concentric folds.

CYG 8 (Photo 16) is comparable to CYG 2 (Photo 10) in that it is predominantly composed of *Smooth III* type with a sprinkling of *Rough* cells.

A fresh experiment was necessitated because some of the giant colonies of the irradiated strains got contaminated. The *Smooth III* colony illustrated in Photo 4 gave a mixed type (Photo 17) just like the control at the commencement of the experiment (Photo 3). The *Rough I* (Photo 5) colony was succeeded on the other hand by a *Rough II* type (Photo 18) at the same time. So, even in the control cultures the changes are not in an identical direction. The colony of CYG 1 (Photo 19) could be classified as *Rough I* but it does not seem to be pure for the type. There are *Smooth* sectors, one of them originating right from the centre. CYG 2 immediately on isolation from plates was predominantly *Smooth III* in character (Photo 10). After about 50 days, it had become a mixture (Photo 20; *cf.* Photo 3). The composite nature of CYG 3 (Photo 11) changes into a *Rough I* type of colony in Photo 21. Mutation seems to have taken place in CYG 4 also. The pure *Smooth I* condition (Photo 12 and 13) has given place to a mixed type in Photo 22. In CYG 5 too, a similar condition is met with (Photo 23). CYG 6 which was originally *Rough I* (Photo 14) has become transformed into the *Rough II* type (Photo 24) after the lapse of about 5 weeks. It shows a few *Smooth* sectors. It is curious that while pure types become mixtures, mixed types become pure. This periodic fluctuation in the population is further evidenced by the changes in CYG 7. The *Rough I* condition illustrated in Photo 15 has given place to the mixed type in Photo 25. The strain CYG 8 which was predominantly *Smooth III* in character (Photo 16) has been replaced by *Rough II* cells (Photo 26).

DISCUSSION

(*a*) *The nature of mutations induced by ultra-violet*

Ultra-violet irradiation is in general assumed to produce gene mutations, while chemical polyploidogens have been used to induce a doubling of the chromosome complement. The results obtained in this laboratory were rather surprising. Exposure to ultra-violet irradiation produced not only gene mutants (Subramaniam and Krishna Murthy, 1948) but also tetraploids (Ranganathan and Subramaniam, 1950). Polyploidogens like acenaphthene (Subramaniam, 1947) and camphor (Ranganathan and Subramaniam, 1950) induced gene mutations apart from a doubling of the chromosome complement. This led to the suspicion that any induction of polyploidy should itself be preceded by a gene mutation. If that is so, the occurrence of polyploidy after ultra-violet irradiation should be as unpredictable as induc-

tion of any specific gene mutation. In accordance with the theoretical expectations, while gene mutations were induced polyploidy did not occur in this series. Gene mutations are reversible. Demonstration of such a reversibility (Subramaniam, Ranganathan and Krishna Murthy, 1948) led to a scepticism regarding the claim of successive mutations described by Skovsted in *Nadsonia* (Skovsted, 1943). At the same time reverse mutations also offered proof for the existence of multiple alleles at the locus governing the nature of sculpturing of the giant colonies. Further support for the above contention is afforded by the results recorded in the present paper.

(*b*) *Effect of ultra-violet rays on higher plants and micro-organisms*

There is a close similarity in the action of ultra-violet radiation on higher plants and micro-organisms. Depending on the dosage, an organism may be killed or a lethal gene mutation producing the same end result may occur. The breakage of the chromosomes and the consequent non-viability of such cells is another factor leading to the death of the micro-organism. Another important factor is, of course, the actual physiological state of the cells at the time of exposure. Fermenting yeast cells which are endopolyploid are on the road to death and final disintegration. Exposure of a purely fermenting culture may not therefore give a correct idea of the lethal dosage.

The prolongation of the lag phase and the persistence of the cells in the prophase condition for a long time has been surmised to be caused primarily by an upset of the nucleic acid cycle (Catcheside, 1948). The breakage of the chromosome at the higher dosage levels is a necessary corollary. A clear distinction has to be made between chromosomal breakages resulting in viable translocations and those in which no such reconstruction is possible. Some of the chromosomal translocation mutants obtained from our control strain by exposure to acenaphthene and thermal shocks have been shown to be viable (Ranganathan, 1950; Ranganathan and Subramaniam, 1950). In fact the evidences indicate that for breakages leading to viable translocation mutants to occur, not only a particular allelic sequence but also one or more specific gene mutations should have preceded a breakage. Exposure of the same strain to the identical agencies after a lapse of 12 to 24 months did not produce the same translocation mutants. The allelic sequences should be in a state of flux owing to the mutations at different loci. A specific mutation leading to a viable chromosomal translocation is surmised to occur only under very definite conditions. Whether exposure to ultra-violet would produce such mutants was also kept in view during these experiments, for the top yeasts so obtained are

not only purely aerobic but excrete considerable amounts of riboflavin (Mitra, 1949). No such top yeast was obtained in the present investigation.

(*c*) *Mode of action of physical and chemical mutagens*

Investigators on chemical mutagens have been struck by the similarity in the action observed to those recorded after exposure to ionizing radiations. Though Dustin (1947) has attempted to classify the chemical mitotic poisons into the colchicine and trypaflavine types, this distinction is found to break down when it is realised that urethane, classified under the latter category, has been shown to be mutagenic (Oehlkers, 1943, 1946, 1949; Vogt, 1948). It appears probable that mitotic poisons may be mutagenic at lower concentrations. Mutagens at higher levels will necessarily have lethal effects. The surprising fact that emerges from the extended observations on the effect of physical and chemical agencies on yeasts is that

Agent	Gene mutations	Chromosomal translocations	Polyploidy	Authors
Acenaphthene	+	+	+	Ranganathan and Subramaniam, 1950
Do. ..			+	Subramaniam and Krishna Murthy, 1949
Do. ..	+			Duraiswami and Subramaniam, unpublished
Thermal shocks	+	+	+	Subramaniam and Ranganathan, 1947; Ranganathan, 1950
Do. ..	+			Subramaniam and Prahlada Rao, 1951
Chrysene ..			+	Mitra and Subramaniam, 1949
Do. ..	+		+	Idem, 1950
Camphor ..	+			Subramaniam and Sreepathi Rao, 1950
Do. ..			+	Ranganathan and Subramaniam, 1950
Ultraviolet ..	+			Subramaniam and Krishna Murthy, 1948
Do. ..			+	Ranganathan and Subramaniam, 1950
Colchicine ..			+	Ranganathan and Subramaniam, 1950

mutagens are capable of inducing polyploidy under specific conditions, while polyploidogens do produce gene mutations. This would become apparent from the above table:—

The only conclusion that could be drawn from the observations tabulated above is that both physical and chemical agencies should have a common mode of action. Such a contention receives further support from published literature. The consensus of opinion is that the observed effects of radiations are due to a transfer of energy to the cell (Catcheside, 1948; Lea, 1946). Auerbach (1949 *a*) considers that there is considerable experimental evidence for Jordon's "Treffergifte" theory according to which chemical mutagens simulate the radiations in that they exert their effects by "localized contacts with certain governing centres of cell activity" (Auerbach, 1949 *a*). What exactly happens when such contacts occur? The retardation of cellular division observed after *x*-irradiation has been attributed to the upset in the nucleic acid cycle of the cell. A dislocation in the normal metabolism of the cell has thus been brought about. On the basis of such an upset, it is possible to understand why ordinary physiological conditions like age or sex of the material should affect the spontaneous mutation rates in animals. Auerbach herself visualizes the possibility that "Treffergifte" agents could act through a change in the metabolic pattern of the test organism. In this connection the results obtained by Wyss, Stone and Haas (1947) are worthy of consideration. They found that when *Staphylococcus aureus* was allowed to grow in a medium containing known substrates which had been subjected to irradiation with ultra-violet, mutation rate to penicillin or streptomycin resistance could be increased. It is presumed that the mutations are caused by the activated chemicals supplied as substrates to the organism. It appears feasible that utilisation of such an activated chemical by the cells will involve a change in the normal metabolic pattern and the observed mutations could therefore be attributed to the upset in the metabolism of the organism. Results obtained by Auerbach (1949 *b*) on the mutagenic ability of formalin also point to such a conclusion.

Such an interpretation would render possible an alignment of the observed effects of cold shock reported by many investigators. Any thermal shock should result in metabolic derangements. Looked at from this angle, the differences between the various chemical and physical agencies would be only one of degree. An upset in the metabolism could then be effected by action either on the nucleus or the cytoplasm.

The underlying causes for spontaneous gene mutations have been a matter of speculation ever since gene mutations themselves were dis-

covered. Attempts have been made to attribute any agency which has been known to accelerate the mutation rate as the primary causative agent. Reasons have been adduced to show that the acceleration in the mutation rate by physical and chemical agencies should primarily be traced to disturbances in metabolism. If the above contention is accepted, any drastic alteration in the metabolic activity of the organism should produce gene mutations.

SUMMARY

1. Investigations in this laboratory indicated that polyploidogens are also mutagenic and that mutagens may induce polyploidy. Doubling of the chromosomes leading to viable tetraploid races is sporadic and such an induction was observed after ultra-violet irradiation.

2. Experiments were therefore carried out to confirm the suspicion that a doubling of the chromosome complement after ultra-violet irradiation may be as sporadic and unpredictable as induction of a specific gene mutation.

3. Photographs of giant colonies of the treated and the control material are presented.

4. The evidence recorded in this paper confirmed the suspicion that induction of tetraploidy being conditioned by a specific gene mutation and induced gene mutations being sporadic, treatment with ultra-violet rays does not always lead to the production of tetraploidy.

5. The existence of multiple alleles at the locus governing the nature of sculpturing of the giant colony is confirmed.

6. Ultra-violet irradiation has immediate as well as delayed effects and the nature of the mutation induced is unpredictable.

7. A theory of the mode of action of the various biologically effective physical and chemical agents on living organisms is considered.

ACKNOWLEDGMENT

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

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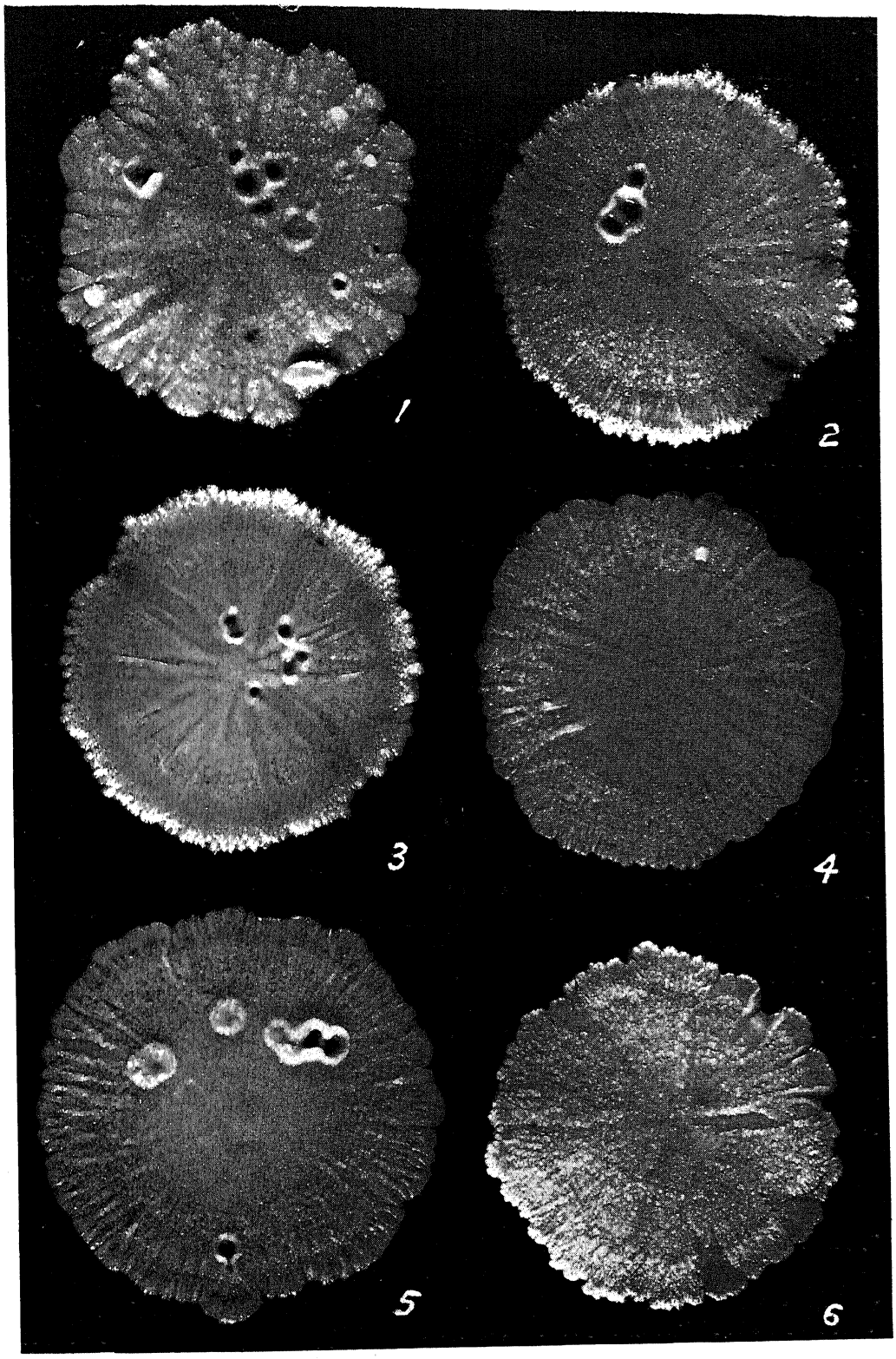
DESCRIPTION OF PHOTOGRAPHS

Photo No.	Strain	Period of growth in days	Diameter of the colony in cm.	Photographed on
1	BY 1	14	3·1	13 -9-1949
2	BY 1S	19	3·1	4-10-1949
3	do.	19	2·6	do.
4	Control 1	13	3·6	13-10-1949
5	Control 2	13	3·2	do.
6	UV 1	19	3·2	4-10-1949
7	do.	20	3·4	5-10-1949
8	UV 2	19	3·4	4-10-1949
9	do.	19	3·5	do.
10	CYG 2	13	3·8	13-10-1949
11	CYG 3	13	3·0	do.
12	CYG 4	13	3·1	do.
13	do.	15	3·0	3-11-1949
14	CYG 6	13	3·3	13-10-1949
15	CYG 7	15	2·6	15-10-1949
16	CYG 8	13	4·0	13-10-1949
17	Control 1	18	2·6	28-11-1949
18	Control 2	18	3·0	do.
19	CYG 1	20	3·9	do.
20	CYG 2	20	3·6	do.
21	CYG 3	20	3·5	do.
22	CYG 4	20	3·3	do.
23	CYG 5	18	2·7	do.
24	CYG 6	21	3·6	1-12-1949
25	CYG 7	17	2·7	28-11-1949
26	CYG 8	16	3·3	9-12-1949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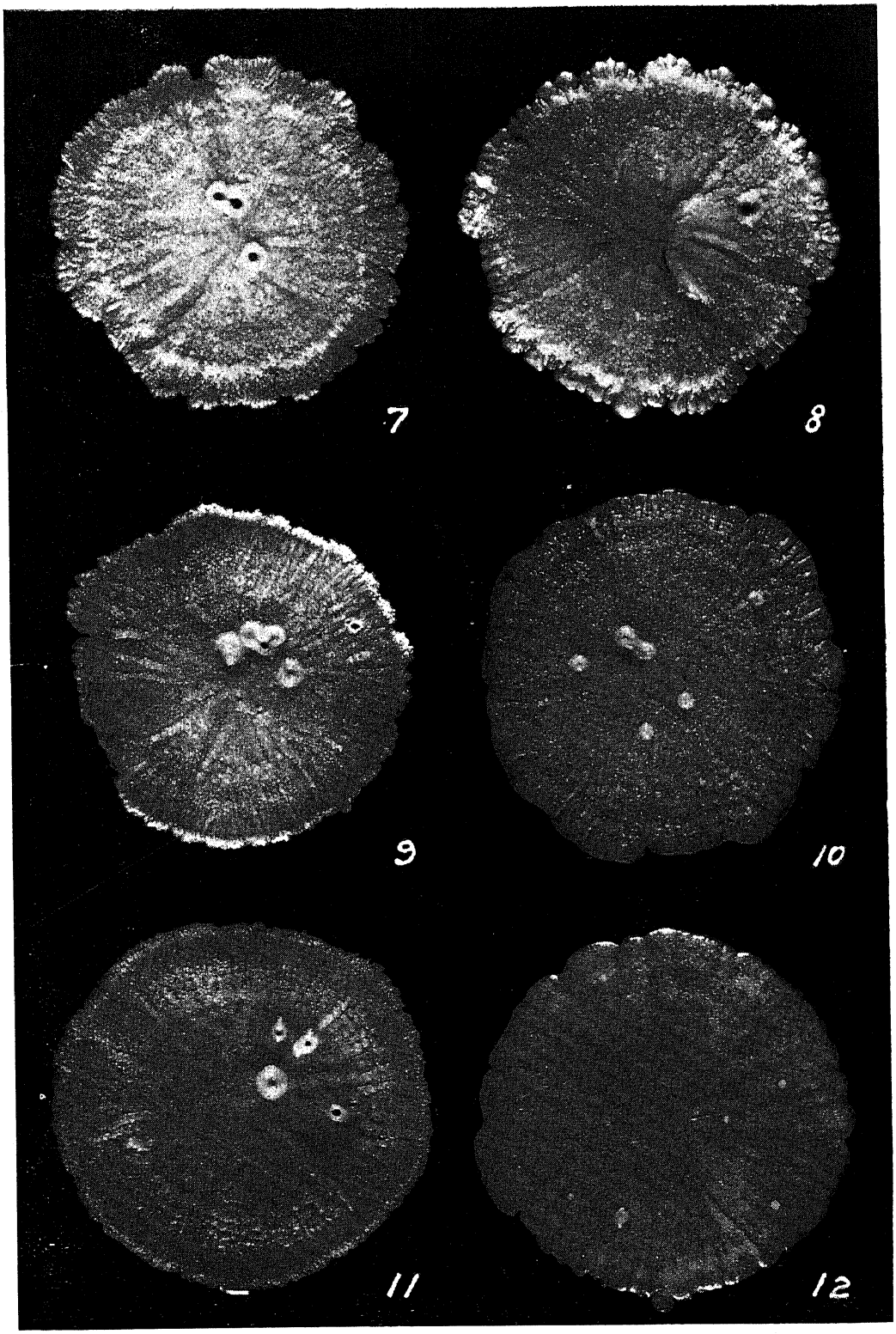
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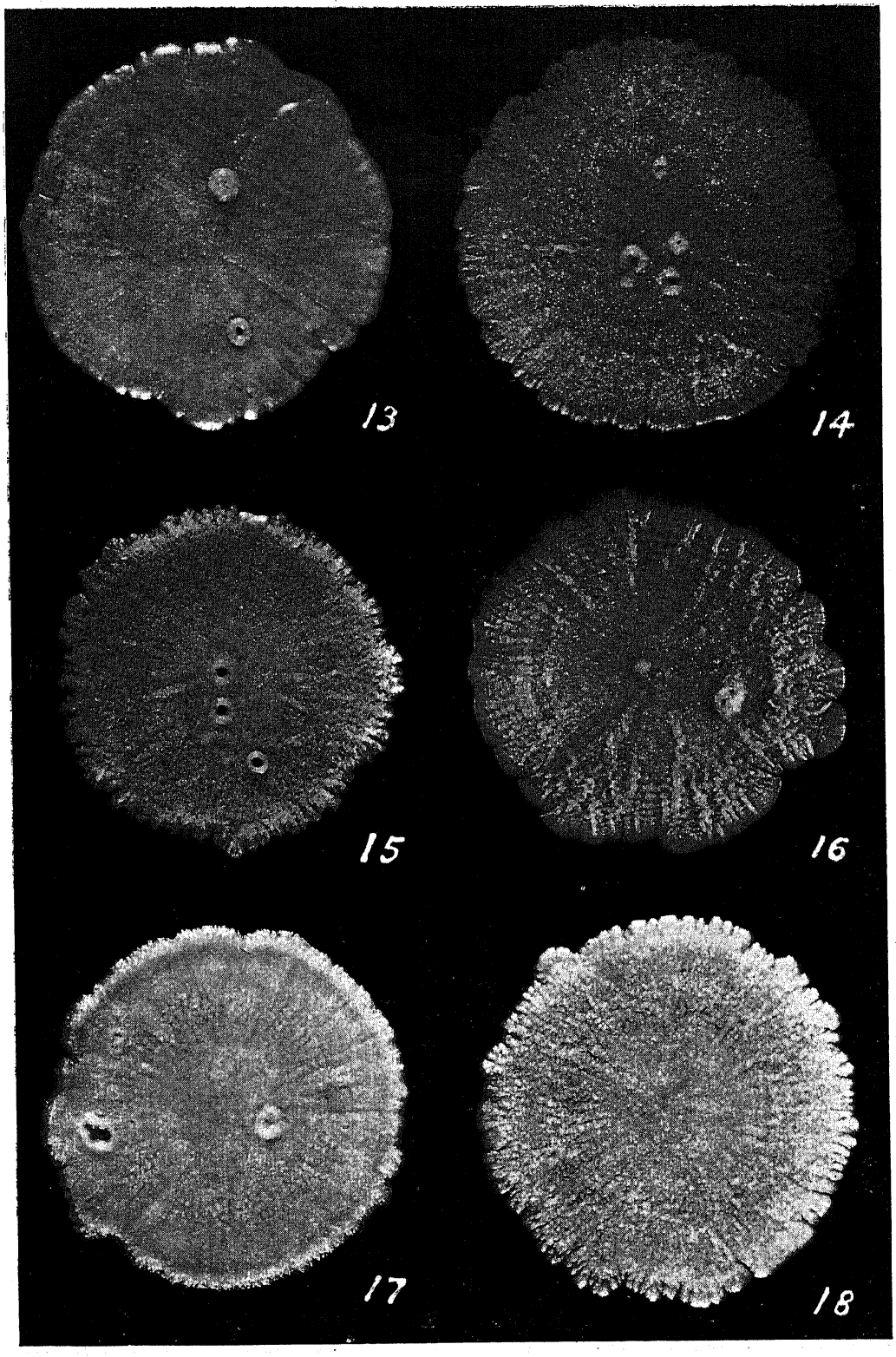
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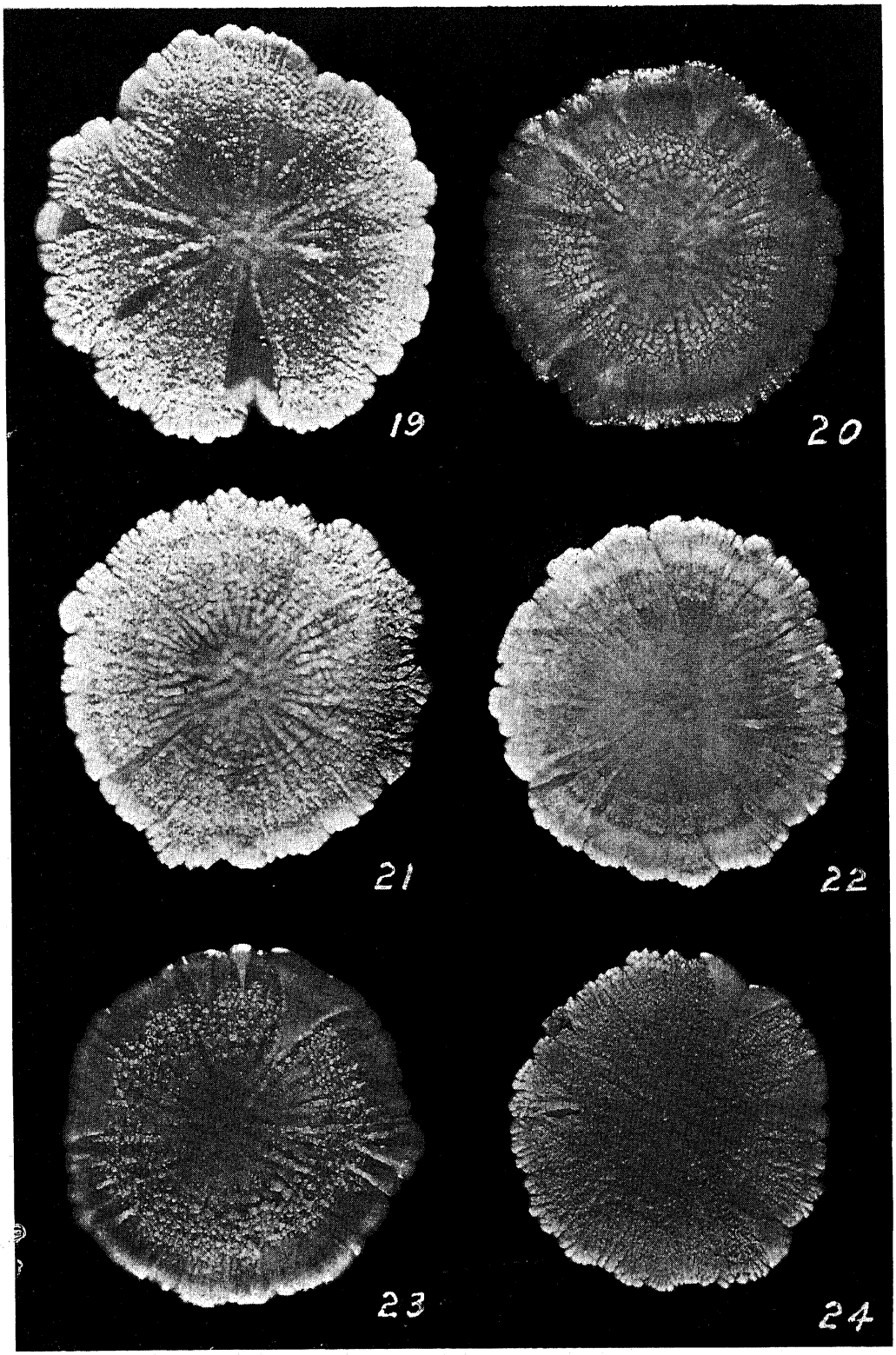
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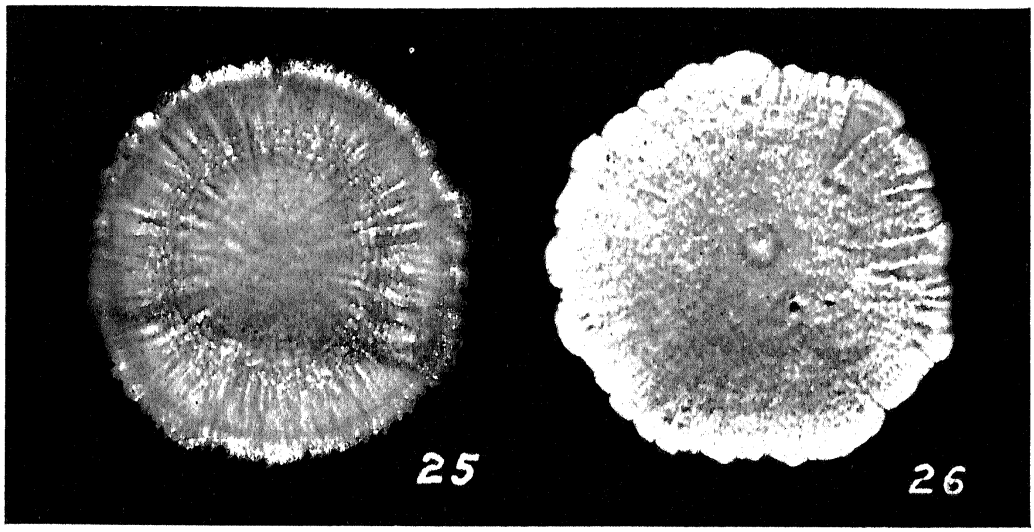
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