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Production of Saltants of *Chaetomium globosum* by Monochromatic Ultra-Violet Irradiation

By

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PLATE XVI

Experiments have been made on the production of mutations by irradiation with monochromatic ultra violet light. The basic object of the experiments was to see whether thresholds exist in this phenomenon of the type familiar in photo-chemical reactions. If such thresholds can be demonstrated, a technique of analysis of biological material might be opened up similar to that used by the physicist in his investigation of atomic nuclei, atoms, and molecules.

Three classes of biological material have been tentatively tried: (i) maize pollen, (ii) *Drosophila* larvae, (iii) spores of the fungus *Chaetomium globosum*—

(i) The work on maize has been given up, as the results have been less encouraging than (ii) and (iii).

(ii) Results of interest have been obtained with *Drosophila* by M. C. Taylor, working in this laboratory. The work is technically difficult, and progress must necessarily be slow.

(iii) A preliminary set of experiments has been completed, in which the spores of *Chaetomium globosum* have been irradiated, and results encouraging from the view-point of the main problem have been obtained.

A stage has now been reached where it appears that the most profitable course is to start afresh, using improved methods suggested by the preliminary experiments. The purpose of the present note is to record a few results of interest that have been obtained during the past year. *Chaetomium* was chosen for investigation because of the work of Dickson (1932, 1933) on irradiation by X-rays and heterogeneous ultra violet light of *C. cochliodes* and other species. *C. globosum*, according to Dickson's results, is the least suitable species, but was used as I was able to obtain it in Australia owing to the kindness of Dr. Waterhouse of the Agricultural Department, University of Sydney, who presented me with a culture.

APPARATUS AND METHODS

A monochromator was constructed in this laboratory, of the general type described by Bowden and Snow (1934), using fused quartz lenses and prisms supplied by Messrs. Sharland, of Thavies Inn, London. The diameter of the lenses is 10 cm., their focal length of the order of 15 cm., and the side of the prisms 8 cm. Most of the work has been done using a mercury vapour arc lamp, but condensed sparks have also been used. Intensities have been measured with a Hilger thermopile.

Spores were spread on uranium glass and irradiated in the focussed spectrum. Intensities available by this method are high, and doses up to 3000 joules per sq. cm. have been given with the 365 $m\mu$ mercury line. The irradiated spores were picked up on a brush and transferred to malt agar in a petri dish. The number of spores in a colony separated in this way was very variable, but was to some extent under control. In the experiments to be described it averaged about 50.

Using the above methods, contamination of the spectral line by scattered radiation was serious, and for this reason the early experiments are not here considered. Later, filters were used with the monochromator, and the results should be trustworthy up to a point, though contamination of 313 $m\mu$ by 302 $m\mu$ and of 334 $m\mu$ by 313 $m\mu$ may still have taken place.

SCOPE OF EXPERIMENTS AND GENERAL RESULTS

Experiments were made with wave-lengths 365 $m\mu$, 334 $m\mu$, 313 $m\mu$, 280 $m\mu$, 265 $m\mu$, 254 $m\mu$, and 230 $m\mu$, but most of the irradiation was given with 254 $m\mu$, 313 $m\mu$, 334 $m\mu$, and 365 $m\mu$. Table I indicates the scope of the experiments. Only those saltants have been included which were successfully separated in an apparently pure state, and remained constant in character for at least five or six sub-cultures.

TABLE I

	Wave-length. $m\mu$	*No. of Colonies Surviving.*	*No. of Saltants Separated.*	*Saltants % of Colonies.* %	*Average Dose Joules/cm.²*
A	254	224	16	7	1·7
	313	163	13	8	142
	334	176	4	2	26·8
	365	239	3	1	515
B	365	180	1	½	2800
Control		396	1	¼	—

It will be noticed in Table IA that the number of saltants produced by 334 $m\mu$ and by 365 $m\mu$ is very much smaller than by 254 $m\mu$ and 313 $m\mu$. The reason for this is quite different in the two cases. Owing to difficulties in irradiating with 334 $m\mu$, the average dose given was much below the optimum, and the corresponding production of saltants was low. This was not the case with 365 mu, and it was suspected that the three saltants that appeared might be due to contamination by scattered radiation, owing to the very long exposures necessary. B shows the result of an experiment made under more stringent conditions to check this point. The irradiation was here carried to a point where about two-thirds of the spores were killed, and the fact that only one saltant appeared from 180 colonies suggests that 365 $m\mu$ kills before it produces saltants. Continuous irradiation for more than a week was necessary.

The one saltant that appeared in control came at the end of the series of experiments, and contributed to the decision to terminate them and start afresh with new material.

ENERGY REQUIRED TO PRODUCE SALTANTS AT DIFFERENT WAVE-LENGTHS

Saltants are produced over a large range of dosage, and there appears to be an optimum value for the number of saltants produced per colony surviving. Doses very near the lethal probably are less effective under the conditions of these experiments than something less than this. An estimate of this optimum from the present experiments may easily be in error by a factor of 2, but, owing to the very large variations of sensitivity at different wave-lengths, it is still of considerable interest.

Table II shows the orders of magnitude of the doses required to produce saltants.

TABLE II

Wave-length. $m\mu$	*Order of Magnitude of Optimum Dose Joules/cm.*^{2}
230	2
254	2
265	2
313	200
334	200
365	2000

Certain points of interest are apparent. All the doses are very large compared with those required to produce other biological effects, which have been measured in this laboratory. They are, for example, at corresponding wave-lengths over 100 times as great as M. C. Taylor has found to be lethal to *Drosophila* larvae, and I have found to be lethal to *Bacillus coli.*

The effectiveness of wave-lengths between 230 $m\mu$ and 265 $m\mu$ is of the same order of magnitude, but at 313 $m\mu$ about 100 times the dose is needed for the same effect. There is no evidence that 334 $m\mu$ is less effective than 313 $n\mu$, but at 365 $m\mu$ very few or no saltants are produced, even with doses ten times as great. It should be noticed that if the insensitivity of this material to irradiation is due to anything in the nature of an impenetrable coat the great difference between the short and long doses would be still greater. There is suggestion of discontinuous increases in efficiency in producing saltants as the wave-length is decreased.

Rough measurements indicate that practically all the radiation falling on a spore is absorbed by it, even at wave-lengths as long as 365 $m\mu$. This leads to the conclusion that at 313 $m\mu$ a region of the order of volume of an atom (say, 3 x 10^{-23} ccs.) absorbs on an average 20 quanta during the delivery of the optimum dose. Even allowing for four saltants out of five to be missed in the subsequent separation, this absorption produces less than one saltant for 100 spores irradiated. The consequences of this inefficiency are suggestive. If it is due to strong absorption in a sheath external to the sensitive region responsible for the production of a saltant, as pointed out above, the difference between the effectiveness of short and long waves must be even greater than the hundred to one given directly by experiment. If there is no protecting sheath, many quantum hits must be made within every atom of the sensitive region before one is effective in producing a saltant.

THE SELECTIVE PRODUCTION OF SALTANTS

In an attack on the main problem, the first effect to be looked for is a selective production of saltants. One might expect greater homogeneity among the saltants produced by long wave-lengths than among those produced by short. The results obtained show that the phenomenon is very complicated, and it is not claimed that a decisive result has been achieved, but the evidence suggests rather strongly that saltants are produced selectively at different frequencies.

The saltant St. II (fig. 1) is very different from the parent. The following may be taken as criteria by which it may be specified. It is sterile under the standard experimental conditions employed. The aerial mycelium is very sparse. The substratum is dark brown, and dark-red hyphae can easily be seen veining the medium, while the parent hyphae cannot be seen in the medium. The growth of the saltant is slower than that of the parent. It forms a dense leathery skin on the surface of the medium with a tendency to wrinkle.

Plate XVI, fig. 1, shows two photographs of the saltant, eight days old, taken by reflected and transmitted light, and comparison photographs of the normal fungus, eight days old, and of *C. murorum*, 13 days old. In many respects St. II resembles *C. murorum* more closely than its parent. *C. murorum* is sterile in the standard experimental conditions.

Table III records the appearance of St. II among the experiments under consideration, also the diversity of types at three different wave-lengths.

TABLE III

Wave-length.	*Total Number of Saltants.*	*Number of Definitely Distinct Types.*	*Number of Occurrences of St. II.*
254	18	13	1
313	13	7	4
334	4	2	3

In view of the small numbers involved, perhaps the fairest way to present the results is to regard the irradiations as grouped into two classes, 'long' 334 $m\mu$ and 313 $m\mu$, and 'short', 254 $m\mu$. St. II appears seven times out of 17 among the long, and once only, and this a doubtful one, out of 18 among the short wave-lengths. The indications are not, of course, that St. II is produced more freely by the long wave-lengths (the doses given are 100 times as great in this region), but that a larger variety of saltants are produced by the short wave-lengths. This is borne out to some extent by the fact that 13 out of 18 of the short-wave saltants are distinct from one another, while only eight out of 17 of the long-wave saltants are of different types.

GENERAL NATURE OF SALTANTS

The following are some of the principal characters that have been observed in the saltants produced in this work.

Saltants are produced with very dense aerial mycelium. In one class this lies close to the medium, giving a matte appearance like filter-paper. In another it is deep as well as dense, completely hiding everything beneath it. The paper-like mycelium may be white or pink, the deep mycelium white or yellow. Both paper-like and deep mycelium may be sterile or carry fertile perithecia. In some saltants the perithecia form on top of the dense, deep aerial mycelium, in some what look like small sterile perithecia without hairs form beneath the surface of the medium. Two saltants form sclerotia. Another shows marked zones, the zoning character appearing in all the progeny, whether grown from mycelium or spores. Perithecia may be yellow-white, blue turning black, or black. All these are fertile, and the spores breed true. There are varying types of infertile perithecia, ranging from bodies looking very like the fertile parent perithecia to small, round translucent bags without hairs. The growth rate varies in different saltants, and may be the same, greater, or less than that of the parent. Plate XVI, fig. 2, shows photographs of a few selected saltants.

IRRADIATION OF SALTANTS AND REPRODUCTION OF THE PARENT FROM A SALTANT

Spores from two of the saltants have been irradiated. Though these saltants were apparently quite stable normally, they both saltated freely when irradiated. This work has not yet been followed up, and only one or two of the secondary saltants have been separated in an apparently pure state. One was apparently identical with the parent (Plate XVI, fig. 3), and has remained so through several sub-cultures. This is in agreement with a similar result observed by Dickson (1932) with *C. cochliodes*, using X-rays. It is, of course, of particular interest as showing that the action of the radiation cannot be looked on as merely destructive. The reproduction of the parent form from the saltant was, in this case, brought about by a wave-length of 230 $m\mu$.

SUMMARY

The following are the most important new facts recorded:—

(i) The optimum dose of ultra violet radiation for the production of saltants in *C. globosum* is of the following order—

Short wave-lengths, 230 $m\mu$ to 265 $m\mu$, 2 x 10^7 ergs/cm^2.
313 $m\mu$, and probably 334 $m\mu$, 200 x 10^7 ergs/$cm.^2$
365 $m\mu$, if effective at all, 2000 x 10^7 ergs/$cm.^2$

(ii) The effectiveness of ultra violet radiation in producing saltants in *C. globosum* is very small (or perhaps zero) at wave-lengths as long as 365 $m\mu$. The ratio saltant-producing effect to lethal effect is smaller than for shorter wave-lengths.

(iii) A saltant produced by irradiation, which was quite stable under normal conditions, saltated freely on irradiation and, among other things, reproduced the parent strain.

In addition to the above facts, it is shown that there is evidence that suggests that saltants are produced selectively by different wavelengths. This is an aspect of the primary object of the investigation, and a fresh series of experiments is planned to follow it up.

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PLATE XVI

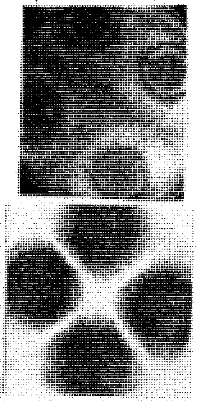
FIG. 1:

- *a.* Four colonies of normal *C. globosum*, 8 days old, reflected and transmitted light. × ⅓.
- *b.* Four colonies of St. II. Four separate saltants produced by 313 *mμ*, 8 days old, reflected and transmitted light. × ⅓.
- *c.* Four colonies of *C. murorum*, 13 days old. × ⅓.

FIG. 2.—Typical saltants produced by irradiation:

- *a.* Produced by 254 *mμ*, 8 days old. Bottom, zoning fungus, produces sparse fertile perithecia after about a month. × ⅓.
- *b.* Four saltants produced by 313 *mμ*, 8 days old. Bottom, fungus bears black perithecia on top of dense, deep, dirty-white mycelium. × ⅓.
- *c.* Saltant, produced by 254 *mμ*, showing knots of sclerotia. It periodically produces dense, white, barren mycelium (see top right). These characters are reproduced in sub-cultures. × ⅓.
- *d.* Two slow-growing saltants produced by 254 *mμ*, 17 days old. Both bear barren perithecia. The branching fern-like mycelium of the top one can be seen under the surface of the medium × ⅓.
- *e.* Very slow growing dense saltant which wrinkles surface of medium, 8 days old. Compare 1*a* normal fungus of same age. × ⅓.
- *f.* Photograph of perithecia on an older culture of e. The saltant comes true from spore or mycelium subcultures. Natural size.

FIG. 3.—The saltant which on irradiation reverted to the parent form. The reversion is in the middle, flanked on either side by colonies of the saltant from which it arose. Natural size.



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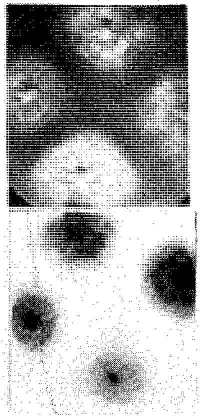

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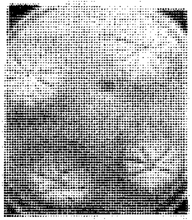

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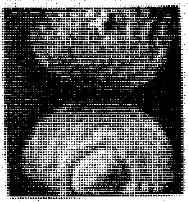

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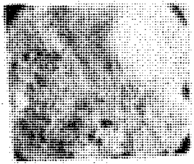

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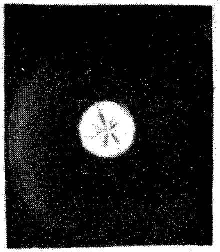

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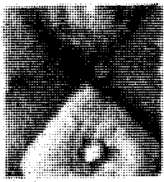

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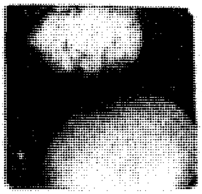

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