THE EFFECTS OF RADIATION ON SOME *POLYPORES* IN CULTURE

BY

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Before I pass on to the subject of my address, I like to place before you a short history of our Indian Botanical Society especially for the information of foreign Botanists gracing this occasion. The Indian Botanical Society was started on December 6, 1920, after deliberations amongst the members of the Botany section of the Indian Science Congress at its Nagpur sitting in consultation with Botanists from different parts of India. The late Dr. Winfield Dudgeon of Allahabad (Jamuna Mission College) took a very prominent part in its inauguration and was its first President in 1921, and the late Prof. S. R. Kashyap of Lahore (Punjab University) was its first Secretary and Treasurer for 1921-1922. The Journal of Indian Botany was started in September, 1919, for publishing botanical work done in India; the Journal owes its inception in the first place to the enthusiasm of the late Mr. L. J. Sedgwick, I.C.S., F.L.S., a keen and prolific worker on Indian systematic botany, and in the second to the kind services of Mr. T. R. D. Bell, C.I.E., the late Chief Conservator of Forests, Bombay, who generously came forward with an offer to guarantee the expenses till the Journal should be so far established as to pay its way; Prof. P. F. Fyson, B.A., F.L.S. the late Professor of Botany of the Presidency College, Madras, was its first Honorary Editor. The Journal was launched with a mixture of hope and doubt; though supported by nearly every important Botanist in India the proposal met with misgivings from several who thought 'the times were yet not ripe'. But within a very short time, in fact in the course of eight months, thanks to the active cooperation of Botanists in India, ten numbers appeared with an average of over thirty pages of original matter and diagrams and about four of abstracts (reviews) to each. The original papers have been on nearly every branch of pure Botany, *i.e.* on Fungi, Algae, Liverworts, Mosses, Gymnosperms, the Taxonomy of flowering plants,

General and Physiological Histology and Morphology, Physiology, Ecology, and a Systematic Flora of a province. Abstracts and reviews have appeared of over 50 papers and books, and occupied 40 pages of small type.

In February 1922, the Indian Botanical Society decided to approach Prof. P. F. Fyson to find out whether he would be willing to turn the Journal over to the Society. After some correspondence, Prof. Fyson finally agreed to hand the Journal over to the Indian Botanical Society. The transfer of the Journal to the Society was finally completed in October 1922 and since then the Journal is the official organ of the Society. It was decided to change the name of the Journal from "The Journal of Indian Botany" to "The Journal of the Indian Botanical Society" in January 1923. No. 6 of Volume III of the Journal was the first number issued by the Society in April 1923. The Journal records the activities of Botanists working in India on various phases of Botany, cytology-papers usually preponderating in number. A booklet on "The Flora of the Indus Delta" by Mr. T. S. Sabnis has been issued by the Society out of the reprints in earlier issues of our Journal. With the transfer of the Journal to the Indian Botanical Society an Editorial Board with an Editor-in-Chief was constituted for conducting the Journal. Prof. Fyson was elected the first Chief Editor of the Journal. He continued as Editor up to 1926, then it passed through the editor-ship of Prof. B. Sahni, Prof. M. O. P. Iyengar and late Prof. S. R. Kashyap in succession. The present Editor is Prof. P. Parija of Ravenshaw College, Cuttack; during his able editorship the Journal has become bimonthly (of about 350 pages a year), while formerly it used to appear quarterly. The total number of members (ordinary and associate) is about 150; the Business-Manager's report gives a detailed account on these points. I am glad to record the good services rendered by Dr. Miss E. K. Janaki Ammal, our present Secretary, to the cause of the Society.

Introduction

Most of the published works on radiation in fungi are of a decidedly qualitative nature without adequate control of environmental conditions, hence it becomes difficult to arrive at a correct interpretation of results which cannot be easily duplicated. The radiation here refers to treatment of malt-extract agar-plate-cultures of three Polypores *(Polyporus ostreiformis, Polystictus leoninus* and *Trametes cingulata)* by ultra-violet rays, X-rays, radium, and sunlight. The effects of radiation, as pointed out by Catcheside (5) are usually manifested in two ways, either (1) by producing *temporary* physiological effects on the individual, *i.e.,* by depressing or accelerating physiological functions or (2) by *permanent* effects leading to death or some modification of the germ-plasm. The first is phenotypic and indirect, producing noxious physical or chemical conditions in the environment of the germ-plasm, and the

second is genotypic, causing direct hits on the chromosomes in the nucleus, a single hit being defined as the absorption of one quantum of energy in the sensitive region. The sequence of events in chemical effects according to G. Failla (10) are (*a*) ionization (*b*) chemical changes (*c*) biological changes; this probably accounts for the delay in the appearance of the effects of radiation in living organisms —known as the latent period.

Most of the effects on fungi are of temporary nature. Saltations or mutations in fungi due to the influence of radiation producing heritable changes are rather few; in the Polypores treated I could not find any. This supports my previous experience in the course of sexuality-study of Polypores (2) that additions of minute doses of poisons and acids or the variations of temperature and of light and darkness and the change of various kinds of nutritive media could not produce any mutation or saltation of the monosporous mycelium, they seemed absolutely stable, not alterable by any change of circumstances or conditions. In this connexion attention may be drawn to the relevant remarks of Burkholder (4) that "in the normal course of events, where light exerts an action upon growth, it is probably brought out by absorbing substances (pigments) normally present in the plant." The three *Polypores* treated here are perfectly white, devoid of any pigmented substance. Even in the case of green plants several investigators have found that different species as well as different individuals of the same species vary in their reaction to the rays; this relates to the question of special sensitivity in some plants. Only those plants whose genes are in an unbalanced state can be easily changed by the action of X-rays, ultra-violet, etc. Radiation-experiments which I have carried on *Polypore*-cultures are summarised below under appropriate headings.

Ultra-violet radiation and solar radiation

Materials and methods

Malt-agar (malt extract 3% and agar 2%) plate-cultures of *Polystictus leoninus, Polyporus ostreiformis* and *Trametes cingulata* were used throughout. The plates were exposed to the full range of quartz mercury arc screened by cellophane paper from a *Hanovia Alpine Sun* operating on D. C. at 3.5 amperes with 100 volts across the arc. The distance of the arc from the culture-plate was in every case kept at 30 c.m., at this distance practically no heating was produced near the culture-plate. The lid of the petri dish was always replaced by a thin cellophane paper (·025 mm. thick) previously sterilised by alcohol, this prevented dust and contamination while allowing U.V. rays to pass through it. The range of wave lengths was from 2400 to 4000 A°u which includes the whole of the biotic and a portion of the abiotic range and some portion of the violet region of the visible spectrum. Two kinds of exposures were given in each case—(1) an almost daily exposure of five minutes' duration for 15 days, (2) and only twice, of fifteen minutes' exposure, in a fortnight.

Results (I) in the case of daily exposure of five minutes' duration for 15 days.

(1) Damage in the main plate of *Polystictus leoninus*

In the case of *Polystictus leoninus* the plate was exposed on 9th October, 1936, just four days after inoculation of the plate so that sufficient space was left in the plate for the growth of the culture following ultra-violet radiation; in this state the vegetative hyphae were all filled up with protoplasm, they were with clamps and with a number of short mediate branches without any trace of basidia.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae The change produced after the very first exposure was considerable damage to the vegetative hyphae. Many become clampless, and protoplasm became broken up into pieces and much vacuolated. These hyphae ultimately lost their protoplasmic contents, became double-walled and were converted into hairs. Apart from mediate branches hyphae without clamps and branched at right angles were also produced in good numbers, but the number of clamped hyphae always preponderated over non-clamped ones. With increasing exposures the non-clamped hyphae were also damaged and showed broken nature of protoplasm. (*b*) Conidia were never produced. (*c*) Chlamydospores were produced in good numbers in cultures one month and ten days old from the date of inoculation.	Basidia (the evidence of fruit-formation) appeared after the third exposure i.e. seven days after the inoculation. The fruiting period in the control plate was also seven days; thus, the initiation of the fruit-formation was neither hastened nor retarded due to radiation. But no porous surface was formed in the irradiated plate nor any regular and erect fruits were produced, as are found in the normal cultures. Smears from the coloured (yellowish) area showed the accumulation of black spots under the microscope within which were found numerous basidia and crystals of calcium oxalate. The basidia were with sterigmata and attached spores, they were irregularly scattered and never arranged in clusters; with increasing exposures the basidia became very much shrunken in appearance.	The upper surface of the irradiated culture gradually developed a dull yellow colour after the fifth exposure which deepened with increased radiation. The yellow colour first developed in smal patches scattered over the surface of the culture and ultimately the whole surface assumed a dull yellow colour, which however was not carried over to its sub-cultures and presented a sort of burnt-up appearance. The hyphae forming the aerial part of the culture plate never tended to go down and sink to the bottom of the medium to avoid strong radiation as has been reported by Weston and Halna ( ). In the course of day-to-day radiation if there was an interruption for two or three days the culture in the main plate showed a slight tendency to reversion to the normal condition.

(2) Damage in the main plate of *Trametes cingulata*

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Slight damage to hyphae was noticed after the second exposure on 10th. November, 1936; the damage became more pronounced with increasing exposures, the hyphae lost their turgid condition and became gradually narrower than the normal ones with their protoplasm either disintegrated or broken up into disconnected masses. Empty hyphae and extremely narrow hyphae were also produced in good numbers and many of them lost their clamps. (*b*) Conidia With increasing exposures the number of conidia decreased till after the fifth exposures on 16th November, 1936 (i.e. 13 days after inoculation) they totally disappeared from the culture. (*c*) Chlamydospores were present in small numbers when exposure was first begun. Their number increased to a maxium up to the seventh exposure (on 23-11-36) after which they gradually diminished but never disappeared altogether; a good many of them were found in empty condition.	This strain of *Trametes cingulata* was highly conidial and non-fruiting, no sporophore appeared even in normal cultures.	No Colouration of the upper surface of the culture plate was noticed as in the case *Polys. leoninus*.

(3) Damage in the main plate of *Polyporus ostreiformis*

In the case of *Polyporus ostreiformis* the strain was non-conidial with a good number of chlamydospores. A smear from the normal culture showed hyphae measuring 4–6μ in breadth with

well-developed clamps and mediate branches in good numbers. During the whole period of the experiment the normal strain was found to fruit only in solid cultures in plates and better fruiting occurred in liquid (malt-ext.) cultures in flasks. No fruiting occurred, however, in tube-cultures. Fruiting occurred in plate cultures in 19–20 days and was confined only on the walls of the plates. In liquid cultures in flasks fruiting was not only earlier but also far bigger in size than in plates. Various methods were tried to induce fruiting in tubes without any success upto January, 1937, but since February, 1937, several tube-cultures which were kept under identical conditions, fruited almost simultaneously, and since then fruiting has always been recorded in tubes.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. No damage was noticed until after the third exposure on 11th. November 1936. Even then the damage was slight and in smears the hyphae were hardly distinguishable from the normal ones. Damage became more pronounced after the seventh and eight exposures and went on increasing with increased doses of radiation. The nature of damage noticed was the same as in other cases. The protoplasm became more and more vocuolated and broken up into disconnected chains with a strong tendency to break up into oidia. Several hyphae lost their protoplasmic contents altogether and a large number of empty hyphae could be found without any clamps. Those that were living became narrower till at the end they became converted into a large number of extremely narrow hyphae containing disconnected masses of protoplasm.	No fruiting ever occurred the irradiated plate but a smear taken 27 days after inoculation from condensed area on the wall of the plate showed the presence of a good number of rudimentary basidia without any sterigmata or spores. No porous area, however, was formed.	Resistence to damage seemed to be more marked in *P. leoninus* or *T. cingulata* and whereas in the latter damage was evident either after the first or second five minutes exposure in *P. ostreiformis* no damage was noticed until after the third exposure. The only noticeable point was the development of a good number of uniformly narrow filled up and elongated hyphae without clamps showing no trace of damage But considering the number of extremely damaged narrow hyphae which still retained clamps at every partition, the number of these narrow healthy hyphae without clamps was not at all greater. Hyphae with clamps always preponderated over those without clamps whether they were filled up or damaged. The number of mediate branches neither increased nor decreased and they were damaged to the same extent as the main hyphae.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) Conidia. A few conidia were developed after the thirteenth exposure on 7th. December, 1936 (34 days after inoculation) but their number never increased. (*c*) Chlamydospores were in fairly good number in the beginning. Their number gradually went on increasing but after nine exposures they rapidly diminished in number but never vanished altogether.		

(1) Changes in sub-cultures from the main plate of *Polystictus leoninus* (*i.e.*, in the first vegetative generation.)

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In *Polystictus leoninus* altogether five subcultures were carried out in malt-agar plates from the irradiated plate, the first after the first exposure, the second after the third exposure, the third after the ninth exposure, the fourth after the fourteenth exposure, the fifth and the last ten days after the fifteenth exposure. Smears from the vegetative area showed hyphae with much broken up protoplasm and much narrower than the normal ones with clamps extremely small and reduced in size. Many of these were ultimately converted into hairs. Hyphae devoid of	The first and the second subcultures fruited after 8 days while the last 3 subcultures fruited on the 9th. day after inoculation. The fruiting period in the control at that time was 6 to 7 days so that fruiting (as noted by the 1st. appearance of basidia in smears) was delayed by 1 to 3 days. Toothed area developed all round the inoculum and smears from these areas showed the presence of numerous basidia arranged in close clusters but very few were with attached spores. The erect fruits that were developed later in these subcultures were found on sectioning to have empty pores in the majority of cases with a few tramal hyphae	

In the vegetative stage.	In the reproductive stage.	Observations.
clamps were produced in good numbers, but hyphae with clamps always preponderated. (*b*) The number of conidia produced was small but later on they disappeared altogether. (*c*) Another feature of these 1st. generation sub-cultures was that chlamydospores were produced in large numbers from the beginning—a condition not found in the normal.	bearing clamps only at places. These fruits on being inserted in agar plates did not shed any spores. The shapes of these fruits were irregular when fully formed.	It is thus seen that the damage due to ultraviolet rays persisted right through the first generation (in plate) and no tendency to reversion was noticed either in the reproductive or in the vegetative phase.

(2) Changes in sub-cultures from the main plate of *Trametes cingulata* (*i.e.*, in the first vegetative generation)

In the vegetative stage.	In the reproductive. stage.	Observations.
(*a*) Hyphae. In the case of *Trametes cingulata* altogether 4 subcultures were carried out from the main plate after the third, fifth, twelfth and fifteenth exposures. The first two subcultures showed slight damage in the beginning but these totally disappeared later, while the last 2 subcultures showed complete reversion to the normal state. Recovery, therefore, occurred in the 1st. generation subcultures. No transfers were made to woodblocks. (*b*) Conidia appeared in large numbers. (*c*) A good number of chlamydospores were present.	No fruiting occurred anywhere even in controls, the strain being non-fruiting.	Damage was first noted on the 11th Nov. 1936, and complete recovery was noted in the 1st. subculture on the 2nd Dec. 1936, within an interval of 22 days. Subcultures were carried upto third generation but in no case the damage was found to persist.

(3) Changes in sub-cultures from the main plate of *Polyporus ostrieformis*. (*i.e.*, in the first vegetative generation.)

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the case of *Polyporus ostrieformis* altogether 4 subcultures were carried from the irradiated plate. The 1st was after the third exposure (just after the damage was noted) on the 11th Nov. 1936; the 2nd after the fifth exposure on the 15th Nov. 1936; the 3rd after the 12th exposure on the 5th Dec. 1936; the 4th and the last after the 15th exposure on the 11th Dec. 1936 (in tube). Smear examination showed that the damage caused by irradiation in the main plate was partly maintained in the 1st generation sub-cultures. Empty hyphae together with a large number of narrow-hyphae retaining extremely narrow clamps and with protoplasm broken up into disconnected masses were found mixed with a large number of healthy hyphae with normal clamps and mediate branches, so that unlike *T. cingulata* recovery in the 1st generation was only partial. A fair number of narrow filled up and elongated hyphae without clamps were found as in the irradiated main plate. (*b*) Conidia developed in small numbers as in the irradiated plate. (*c*) The number of chlamydospores always remained a few.	No fruiting occurred in any subculture.	The development of conidia in small numbers in these subcultures lends support to the persistence of the damage.

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus leoninus* in order to find out whether the damage in the first generation persisted and also to study the subsequent nature of growth, the last three subcultures of the first generation were again subcultured in malt-agar tubes on 1st December, 1936. These three subcultures constitute the second generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Smears from these tube subcultures showed a strong tendency to revert to the normal. In the first few smears damaged condition of hyphae was only slightly perceptible but this damage totally disappeared afterwards and complete reversion to the normal condition was found in this second generation. The damaged hyphae that were occasionally found formed nothing but a stage in the conversion of healthy living hyphae into dead double walled hairs as are found in old normal cultures. Hyphae were all with clamps and with mediate branches arising at right angles here and there, broad like the normal ones, with well-developed clamps. The broken up or vacuolated nature of the protoplasm totally disappeared. Out of the five sub-cultures from the main plate the last three were subcultured in wood block on thirteenth November, 1936. The woodblock cultures, therefore, represent the second generation. The wood	Fruits appeared in all these three sub-cultures after seven days which was also the fruiting period for the normal ones at the period. These fruits were quite normal with regular pores which showed in section basidia in close clusters bearing sterigmata and normal spores and were found intermixed with tramal hyphae bearing clamps but no spores (cystidia-like bodies.) Spore-fall was copious on agar plate whence they were transferred to malt-agar tubes and kept in incubator at 37°C owing to the then low temperature condition. The spores started germinating four days after they were transferred and regular fruits appeared after eight days (the normal period).	The woodblock cultures were made at a time when damage was fully persisting in plates. But complete recovery to the normal condition was noted on the fourteenth January 1937 i. e., forty-five days after wood was inoculated while fruiting occurred in all between 66-72 days after the inoculation i.e. between 21-27 days after recovery was noted, while the control culture on wood-block fruited between 14-36 days. The fruits formed were all very regular and all shed spores copiously in agar-plate on the next day after insertion. These spores germinated almost simultaneously and produced clamps after five days in agar plate. They were not transferred to malt agar tubes. The malt agar tube sub-cultures of the second generation were transferred to the third, fourth and fifth generations in tubes. In no case was any damage found to exist and fruits always occurred within the normal period, which was extended owing to the

In the vegetative stage.	In the reproductive stage.	Observations.
used was that of mango. Growth was very vigorous in wood and the very first smear examination on the fourteenth January, 1937 showed complete recovery of the vegetative hyphae to the normal state. (*b*) Conidia were very rarely met with. (*c*) Chlamydospores developed in small numbers in malt-agar tubes but they were not found in wood-block cultures.		low temperature conditions during the winter (10-12 days).

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(2) In the case of *Polyporus ostreiformis* the first three sub-cultures of the first generation were again subcultured in tubes and complete recovery of vegetative hyphae was noted in them.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damaged nature of hyphae totally disappeared and smears showed normal hyphae with clamps and mediate branches. (*b*) Conidia were not found in any of them or in any subsequent generation. (*c*) Chlamydospores developed in good numbers.	No fruits appeared either in the second or third generation-sub-cultures as they were all in tubes (not fruiting in tubes then)	Damage was first noted in main plate on eleventh November, 1936 and complete recovery was first noted in tubes of the second generation on twelfth December, 1936, the intervening period being 31 days. Subcultures were carried upto the third generation but no damage was found to exist. No transfers were made to wood-blocks.

Recovery from damage

In the case of *Polystictus leoninus* damage was first noted in the plate on 9th October, 1936, after the first exposure. It persisted throughout the first generation in plate subcultures and complete

recovery was noted in tube-subcultures of the second generation on 9th December, 1936. The total period was therefore 60 days. In wood-block cultures of the second generation the recovery period calculated from the date of inoculation of the wood was 45 days. The fruiting period of the main plate and of subcultures of *P. leoninus* is summarised below:—

The main plate fruited in the course of 7 days. But no regular fruit-body or fruiting areas were formed.		Controls also fruited on the 7th day.
	Sub-cultures / Fruiting period	
No regular fruits formed in (1) and (2)	(1) 1st generation fruited in 8—9 days.	Control fruited in 6-7 days
	(2) 2nd. generation ,, ,, 7 days.	Control ,, on the 7th day.
Regular fruits formed in (3) (4) and (5)	(3) 3rd. generation ,, ,, 10 days.	Control ,, also on the 10th day.
	(4) 4th. generation ,, ,, 12 days.	Control ,, also on the 12th day.
	(5) 5th. generation ,, ,, 10 days.	Control ,, also on the 10th day.

Recovery from damage in *Trametes cingulata* was noticed in the first generation-subcultures and that in *Polyporus ostreiformis* in the second generation.

Results (II) in the case of only two exposures of fifteen minutes each in a fortnight.

Damage in the main plate of *Polystictus leoninus*

(1) In the case of *Polystictus leoninus* two exposures were altogether given, each of 15 minutes' duration. The first exposure was given on 20th October, 1936, *i.e.,* 5 days after inoculation—immediately preceding the usual fruiting date and the second exposure on 5th November, 1936, *i.e.,* 15 days after the first exposure. The irradiated plate together with a control was kept in diffuse light. No heating was noted near the culture even after 15 minutes' exposure. The vegetative hyphae were all healthy and there were no basidia when exposure was first begun.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. After the first exposure dead empty or partially empty hyphae, some even with empty	Fruiting was retarded to a much greater extent than in the previous experiment. The first appearance of basidia was noted after	No tendency of the mycelial mat to go down to the bottom of the petridish was noted, neither was there any indication of

In the vegetative stage.	In the reproductive stage.	Observations.
clamps still attached, were found in large numbers and living hyphae altogether lost their healthy appearance and the protoplasm became broken up into chains of oidia showing a tendency towards haploidisation. During the next 15 days that intervened before the second 15 minutes' exposure, the vegetative growth was rather slow and the hyphae produced were both healthy or damaged in appearance. But the healthy hyphae, though filled up with protoplasm as those of the normal ones, were all much narrower than the normal ones and clamps much reduced in size. Hyphae without clamps which had the protoplasm disintegrated, were produced in large numbers but these were always exceeded by the numbers of hyphae with clamps which showed similar appearance. Many of these were found to be converted into dead, double-walled hairs. (*b*) Conidia were not found in the irradiated plate. (*c*) Chlamydospores appeared quicker (after 20 days) than in the case of daily 5 minutes exposure. The number of chlamydospores went on increasing from day to day till the second exposure after which a sharp decline in their number was noted. A large number of chlamydos-	15 days in smear only while the control fruited perfectly well after 8 days. Thenceforth, basidia could occasionally be found in smears, they never arranged in close series but always irregularly scattered and only rarely they were found with attached spores. Furthermore, the basidia were all much shrunken in appearance and much smaller in comparison with normal. No porous surface appeared and therefore, toothed areas were entirely absent. No outward indication of fruiting could be found from the appearance of culture as a whole except for the presence of one small area forming a yellow spot. This developed nearly after 25 days and did not develop any spores.	reversion to normal conditions in the main plate. The colour of the mycelial mat never changed from white to dull yellow or dirty yellow as was found in the previous experiment with the main plate.

In the vegetative stage.	In the reproductive stage.	Observations.
pores were killed by the second exposure, and some became empty, though living chlamydospores still persisted in good numbers. But these gradually diminished in number and almost vanished later.		

Damage in the main plate of *Trametes cingulata*

(2) In the case of *Trametes cingulata* the exposure was given on 10th November, 1936, and the second exposure on 26th November, 1936. Altogether two exposures were given, each of 15 minutes' duration.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The changes produced in the main plate were the same as in the previous experiment. The same nature of damaged hyphae was found. The second exposure only served to enhance the damage already present. (*b*) Conidia were enormous in the beginning, but they slightly diminished till the second 15 minutes' exposure. The conidia disappeared altogether after the second exposure of 30 minutes. (*c*) Chlamydospores were present in small numbers throughout but they too disappeared altogether after the second exposure.	No fruit formed even in the control, as it was a non-fruiting strain.	Damage became evident after the first exposure on 10th November, 1936 and no sign of recovery was noted during the 15 days that elapsed before the second exposure.

(3) Damage in the main plate of *Polyporus ostreiformis*

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the case of *Polyporus ostreiformis* damage was evident after the very first exposure. A good number of hyphae were killed and emptied out and many of the living hyphae showed protoplasm broken up into disconnected chains. During the fifteen days that followed before the second fifteen minutes, exposure a slight tendency to revert to the normal state was noted in the irradiated plate, as the number of healthy clamped hyphae slightly increased from day to day. The second exposure caused considerable damage to the vegetative hyphae as healthy hyphae almost disappeared from the culture. Many more hyphae were emptied out and lost their clamps altogether; out of the living ones a good number was found to undergo disintegration. After the second exposure a good number of long, uniformly narrow and filled-up hyphae without clamps developed mixed with damaged clamped hyphae. Clamped hyphae that remained were all damaged in appearance and were either extremely narrow or some as broad as normal. The number of mediate branches	Fruit appeared in the irradiated plate on 25th. November, 1936, i. e. 20 days after the inoculation and immediately preceding the second exposure on 26th. November 1936, on the wall of the plate. The control plate fruited one day earlier on 24th. November 1936. The fruit in the irradiated plate showed regular pores and a section showed pure basidia in dense layers in each poretube, bearing sterigmata and spores exactly similar to the normal spores in length and breadth. The fruit was inserted in agar plate on 12th December, 1936. Sporefall was copious and continued for three days. The agar plate was kept inside the incubator. Germination was quick and in normal manner and a smear-examination after five days showed normal hyphae of *Polyp. ostreiformis* with clamps, mediate branches and chlamydospores.	As the fruit was formed after the first exposure it is evident, therefore, that continuous exposure for 15 minutes caused damage only to the vegetative phase and did not at all affect the reproductive phase in *Polyp. ostreiformis*.

In the vegetative stage.	In the reproductive stage.	Observations.
neither increased nor decreased. (*b*) Conidia, a few in number were developed on 2nd December, 1936 after the second exposure (27 days after inoculation) but they never increased. (*c*) Chlamydospores were in good numbers in the beginning but they diminished in number after the second exposure and never vanished altogether.		

Changes in subcultures from the main plate (*i. e.* in the first vegetative generation)

(1) In the case of *Polystictus leoninus* altogether three sub-cultures were carried out from the main plate. The first subculture was after the first 15 minutes' exposure, the second after the second 15 minutes' exposure, and the third 20 days after the second exposure. Of these three subcultures, the first and the second were in plate while the third was in tube.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The characters of the first two subcultures were almost identical, but the third subculture differed in many respects. In the first two subcultures the damaged conditions of the vegetative hyphae fully persisted. They were all much narrower than the normal ones with clamps extremely small and reduced in size. Protoplasmic discontinuity of the hyphae with a strong tendency to liberate oidia was evident.	Fruits appeared in all after 9 days, while the fruiting period in the control (at that time) was 6-7 days, so that fruiting was delayed by 2-3 days. The fruits on sectioning showed regular pore-tubes within which were found a large number of basidia in series with attached basidiospores, probasidia and tramal hyphae bearing clamps only (cystidia-like). The fruits of the third subculture in sections showed regular pores containing basidia,	It is thus seen that though considerable damage persisted in two plate sub-cultures of the first generation a small amount of reversion to normal state was also noticeable in the first generation since (a) the condition of the vegetative hyphae in the tube-sub-culture was almost like that of the normal and the damage was only slight; and (b) the fruits of the first generation (both in plate and tube) developed regular pore-

In the vegetative stage.	In the reproductive stage.	Observations.
Clamped and non-clamped hyphae were both present; sometimes non-clamped ones were in excess of the clamped ones while at other times the clamped ones were greater in number. Mediate branches were always present and conversion of living hyphae into hairs was noticeable to the same extent as in the main plate. Another feature was that the damaged condition of hyphae was always more pronounced in the second subculture (made after the second exposure) than in the first one. The third subculture in tubes twenty days after the second 15 minutes exposure was quite unlike the other two in that the damaged condition of the vegetative hyphae was only slightly perceptible. Damaged hyphae were sometimes found but the majority of the hyphae were much healthier though still much narrower than the normal ones. Majority were filled up and mostly arranged in parallel rows in smears. (*b*) Conidia in the first two subcultures were almost none, one or two being very rarely found, while in the third subculture conidia were in fairly good number. (*c*) Chlamydospores in the first two subcul-	basidiospores and tramal hyphae elongations. Two fruits from the second subculture and another from the third subculture were inserted in agar plate on 12th December, 1936. Spore-fall was scanty on the next day from both but spores did not germinate even after 7 days. As it was thought that germination was checked owing to crowding of spores in a limited space, spores were transferred from both to malt agar tubes and kept inside incubator (37°C) owing to low temperature conditions at that time together with the agar-plate containing spores. But none of the spores germinated. The spores were not, therefore, viable.	tubes, containing basidia and basidiospores though the latter were non-viable.

In the vegetative stage.	In the reproductive stage.	Observations.
tures never developed in large numbers and their number remained a few throughout; a peculiar feature was the formation by some cystidia-like chlamydospores. But in the third sub-culture chlamydospores developed enormously.		

(2) In the case of *Trametes cingulata* three subcultures from the main plate were carried out, the first after the first 15 minutes' exposure, the second after the second 15 minutes' exposure and the last on the twelfth day after the second exposure. Complete recovery to the normal state was noted in all of them. Subcultures were carried up to the third generation and in no case damage was found to persist. Damage was noted first on 10th November, 1936, and recovery on 23rd November, 1936, thus the intervening period was 13 days. Exposures given daily for 5 minutes are, therefore, more injurious than a single long exposure twice in a fortnight. No transfers were made to wood-block and fruiting occurred anywhere even in the control.

(3) In the case of *Polyporus ostreiformis* three subcultures were carried out from the irradiated plate, the first after the first 15 minutes' exposure, the second after the second 15 minutes' exposure and the third and the last nine days after the second exposure. The first two subcultures were in plates but the last one was in tube and hence no question of fruiting can arise in the case of the last subculture. But while the last subculture showed *complete* reversion to the normal state, the first and the second subcultures showed some differences.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In the first subculture the hyphae were slightly damaged and majority were healthy, while in the second ones majority of the hyphae were damaged and only a few were healthy.	No fruiting occurred in the second sub-culture (i.e., after 30 minutes's exposure). But fruiting occurred in the first subculture (i.e., after 15 minutes's exposure) within the normal period—nineteen days after inoculation. The fruit was	Only the last subculture showed *complete* reversion to the normal state.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) No conidia were present in the first sub-culture, but a few conidia could be found in the second. (*c*) Chlamydospores were a few in the first sub-culture but always greater in number in the second.	well-formed showing in cross-section pure basidia bearing sterigmata and spores fully agreeing with the normal ones in measurement. The fruit was inserted in plate on 23rd December, 1936, and spore-fall occurred within four hours and continued for five days. The spores germinated quickly inside the incubator at 37° C. in normal manner and clamps developed after five days in agar plate.	

Changes in the second set of sub-cultures in tubes from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus leoninus* as growth became checked in both plate and tube-subcultures they were transferred on 2nd December, 1936, to fresh malt-agar tubes. They formed the second generation. The damage totally disappeared and complete reversion to the normal state took place in the second generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The hyphae were all like the normal ones and were filled up with well-developed clamps and mediate branches. The three subcultures from the first generation (in plate) were transferred to wood-blocks (mango) on 11th November, 1936. Growth was very vigorous and smear examination on 2nd January, 1937 showed complete recovery in wood-blocks too. Recovery, therefore, took place after thirty-three days in all.	In malt-agar tubes fruits appeared on 9th December 1936 within the normal period (7 days). The fruits on sectioning showed basidia with attached spores mixed with tramal hyphae bearing clamps and some even bearing terminal mature spores. Three fruit each from one sub-culture were inserted in agar plate on 18th December, 1936. Spore-fall occurred on the next day from all but their number was scanty in comparison with the large number of spores discharged	Sub-cultures were carried upto the fifth generation. Damaged condition was never found and fruiting always occurred within the normal period which ranged from 10 to 11 days.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) In malt-agar-tubes no *conidia* were produced as in the case of normal growth, but in wood block cultures a small number of conidia was found. (*c*) In both cases (malt-agar tubes and wood-block culture) a few *chlamydospores* were found.	from a normal fruit. However, the spores all germinated inside the incubator after four days whence they were transferred to malt-agar tubes. Among the wood-block cultures fruit formation occurred in the first subculture on 10th April, 1937, after 120 days; in the second subculture on 21st April, 1937, after 131 days; and in the last subculture on 4th February 1937 after 55 days. The fruiting period, when calculated from the date of recovery, was as follows:— First subculture 87 days Second subculture 98 days Third subculture 22 days Control 14-36 days The fruits were all well-formed with regular pores and were inserted in agar plate. Spore-fall was obtained by the next day upon agar plate, spores germinated in normal manner and clamps developed after five days in agar plate. They were not transferred to malt-agar tubes.	

(2) In the case of *Polyporus ostreiformis* though complete recovery was noted in the last subculture (in tube) of the first generation, recovery was partial in the first two subcultures. They were, therefore, again subcultured in tubes on 8th December, 1936, and complete recovery was noted. But as they were in tubes and not in plates, no fruit appeared. Subcultures were carried upto the third generation in tubes and no damage was noticed in any case. No transfers were made to wood-block.

Recovery from damage

In the case of *Polystictus leoninus* damage was first noted in the main plate after the first exposure on 21st October, 1936. The damage persisted in plate subcultures of the first generation. Complete recovery was noted in tube subcultures of the second generation on 9th December, 1936. The total period was 44 days. The recovery-period in wood-blocks (which represent cultures of the second generation) calculated from the date of inoculation of the wood was 33 days. Daily five minutes' exposure is, thus, more injurious than one long exposure given twice. Ramsey and Bailey (13) also hold that "increasing the number of exposures is more effective than increasing the length of exposure". The fruiting period of the main plate and of the subculture of *P. leoninus* is summarised below :—

	The main plate fruited in the course of 15 days. But no regular fruit-body or any fruiting areas nor any spores were formed, only Lasidia had appeared.		Control fruited in 8 days
	Subcultures	Fruiting period	
No Regular fruits formed in (1) & (2)	(1) 1st. generation	9 days	Control fruited in 6 - 7 days
	(2) 2nd. generation	7 ,,	Control fruited in 7 days
Regular fruits formed in (3) (4) & (5)	(3) 3rd. generation	10 ,,	Control ,, 10 days
	(4) 4th. generation	11 ,,	Control ,, 11 day
	(5) 5th. generation	10 ,,	Control ,, 10 days

Recovery from damage in both *Trametes cingulata* and *Polyporus ostreiformis* was noted in the first generation subcultures. In *Polyporus ostreiformis* damage was first noticed in the main plate after the first exposure on 11th November, 1936. Complete recovery was noted in the third tube-subculture of the first generation on 6th December, 1936. The interval, therefore, was 25 days, which is quicker than in the previous case.

Leaving aside fruit-formation in the main plate which occurred after the first exposure of 15 minutes at a time when there was no great damage to the hyphae, fruit-formation in the first subculture from the main plate deserves notice. This subculture was carried out when the main plate had received continuous exposure for 15 minutes and the fruit formed was normal in every respect. But the corresponding subculture in the previous experiment after three consecutive exposures of 5 minutes each, showed no fruiting. It is, therefore,

evident that even as far as fruit-formation is concerned (*i.e.*, in the reproductive phase), exposures of 5 minutes' duration given on three successive days are more injurious than a single long exposure of equal duration. This is further supported by the fact that in every case studied recovery in the vegetative phase was quicker in the case of twice 15 minutes' exposure in a fortnight than that of daily 5 minutes' exposure for 15 days. This statement applies equally well to *P. leoninus, T. cingulata* or *P. ostreiformis.*

Irradiation of fresh spores of *Polystictus leoninus* by ultra-violet rays (a) for thirty minutes (b) ten minutes and (c) five minutes.

(*a*) A sporophore of *Polystictus leoninus* from a tube culture was inserted on agar plate on 27th January, 1937. Spore-fall occurred within four hours of insertion and was collected in two plates. One plate containing fresh ungerminated spores was irradiated by ultra-violet rays through cellophane paper (·025 mm. thick) on the same day for a period of *thirty minutes* while the other plate was kept as a control. The type of lamp (Hanovia Alpine Sun), the distance from the arc etc. were the same as in the previous experiments. Both the irradiated and non-irradiated plates were kept inside the incubator at 37° C. The control spores germinated within three hours while the irradiated spores did not germinate even after eight days.

(*b*) Another agar plate containing fresh spores of *Polys. leoninus* dropped from an artificial fruit on 3rd February, 1937, was similarly irradiated for *ten minutes* keeping a control plate. Both the plates were kept inside an incubator at 37° C. The spores in the control-plate all germinated by the next day while the irradiated spores did not germinate even after eight days. Some of the irradiated spores were transferred to malt-agar plate, but here they also did not germinate.

(*c*) On 23rd February, 1937, fresh spores of *P. leoninus* in agar plate were similarly irradiated for five minutes only, keeping a control plate. Both plates were kept as before inside the incubator at 37° C. The spores in the control-plate all germinated by the next day while a good number of irradiated spores germinated only after two days, *i.e.*, on the 25th February; they were both subcultured to malt-agar tubes and were kept in the culture room. Growth was vigorous in the tubes containing normal (non-irradiated) spores and perfect fruits appeared in the course of seven days while the tubes containing irradiated spores showed no sign of progress at all even after sixteen days.

On the same day fresh spores in an agar-plate from an artificial fruit of *P. leoninus* in a tube culture after complete recovery from the effects of radium (fruit of the third subculture in the fourth generation from the main plate of *P. leoninus* exposed to 120 mg. of radium for six days) were irradiated for five minutes only,

keeping a control-plate. The spores in the control-plate all germinated by the next day while the irradiated spores germinated two days later. Both were transferred to malt-agar tubes and were kept in the diffused light of the culture-room. As in the preceding case growth was vigorous in the tube containing non-irradiated spores, leading to the formation of perfect fruit in the course of seven days, but the tube containing irradiated spores did not show any trace of growth even after sixteen days. Thus, it is seen that spores dropped from a sporophore of *P. leoninus* in malt-agar tube after complete recovery from treatment with heavy doses of radium reacted to ultra-violet rays in the same way as spores from a normal fruit-body in culture.

Side by side, the influence of sunlight, incandescent light (150 C. P. bulb) and of heat upon cultures of these Polypores was studied.

Influence of sunlight exposure for ten days on culture of (a) Polystictus leoninus.

A plate culture of *P. leoninus* inoculated on 25th November, 1936, was exposed to solar radiation. The petri-dish containing the culture was placed on a thick glass plate over which was placed a belljar the rim of which was sealed to the glass surface with vaseline. It was exposed to the direct rays of the sun over a raised horizontal wooden platform. Exposure was first given on 28th November, 1936, *i.e.,* three days after the inoculation and ended on 9th December, 1936. During this period exposures were given almost daily for 10 days. The period of exposure was four hours from 12—4 P.M. The temperature of the surrounding air varied from 30°–33° C. Two subcultures were carried out, the first after 3 days' exposure and the second after 6 days' exposure.

Results in the main plate

The culture media contracted from the edges of the plate and gradually came to occupy the centre with increasing evaporation of moisture, it became reduced to a thin sheet and presented a dried and hardened appearance. The result produced by solar radiation was much more significant than that produced by ultra-violet, X-ray or radium exposures. The very first exposure on 28th November, 1936, was sufficient to check the growth of the colony. No further growth occurred during the period before the second exposure and growth became permanently checked. The fluffy nature of the hyphae became entirely lost and the whole hyphal mat presented an adpressed appearance but did not tend to sink down to the bottom.

With increasing exposures the hyphae became extremely narrow with clamps extremely reduced in size. The protoplasm became broken up into disconnected masses with a tendency to break up into oidia. A few conidia developed after the third exposure on 2nd December, 1936 *i.e.,* 4 days after inoculation but they never increased. Furthermore, the number of mediate branches became

very much reduced. A large number of hyphae became totally clampless and such non-clamped hyphae came into preponderance. Hyphae were also found to disintegrate in large numbers. No chlamydospores were produced. There were no basidia when the culture was first exposed, and no basidia ever developed in the main plate, so that fruiting was entirely suppressed. In other words, the culture ultimately became dead. Hot-agar was poured over the culture on 15th December, 1936 but there was no revival.

Results in the sub-cultures (*i.e.*, in the first vegetative generation)

Two subcultures were carried out in malt-agar tubes after the third and sixth exposures, but no growth was noticed at all either at room temperature or in incubator at 37° C.

Influence of sunlight-exposures for fifteen days on culture of (b) *Trametes cingulata* :—

A petri-dish culture of *T. cingulata* inoculated on 4th May, 1937, was exposed after six days to direct solar radiation. The plate was almost full when the culture was first exposed. Exposures were given for three hours daily for 15 days, the first exposure being given on 10th May, 1937, and the last on 26th May, 1937.

The time of exposure was from 9 A.M.–12 P.M. The temperature of the surrounding atmosphere varied during this period from 35°–40° C. The method of exposure was the same as in the previous experiment, excepting that the sides of the petri-dish were covered by a strip of white paper, and an Erlenmeyer flask containing 4% alum solution was placed over the petri-dish inside the belljar. This devise eliminated a certain amount of heat and the temperature taken by actually inserting a thermometer inside the petri-dish during exposure was found to be 30°–35° C. which was less by 4 to 5 degrees than that of the surrounding atmosphere and higher by 1 or 2 degrees than the room temperature.

Results in the main plate

(1) The first effect was a change in the external appearance of the culture. The culture media together with the hyphal culture contracted gradually from the circumference of the petri-dish and ultimately came to occupy about three-fourths of the plate owing to evaporation of moisture from the medium. The thickness of the medium was also very much reduced. The plate showed distinct zonation before exposure—the effect of alternation of light and darkness—but the difference between zoned and non-zoned areas ultimately disappeared and the culture presented an adpressed appearance.

(2) Damage to vegetative hyphae was rather insignificant in comparison with *P. leoninus*. A good number of dead and empty

hyphae were produced. The protoplasm of vegetative hyphae became broken up into disconnected chains and a strong tendency to break up into oidia was noticed at places only. But the majority of the hyphae were healthy though a little narrower than the normal ones.

(3) The number of conidia slightly increased at first but after the third exposure, they *rapidly diminished* in number as the majority of them became converted into *thick-walled chlamydospores*. Chlamydospores ultimately preponderated over conidia.

(4) Mediate branches, in addition to those already present, were found to increase. They were not produced from the main hyphae, but were developed as direct prolongations of the clamps. They were short in length, without any clamps, and did not present a damaged appearance. This is usually regarded as reduction to the monocaryon stage, as pointed out by Brodie (3). No fruit appeared as it was a non-fruiting strain.

Results in the sub-cultures (*i.e.*, in the first vegetative generation)

Three subcultures were taken from the main plate, the first after the second exposure on 12th May, 1937, and the second after the seventh exposure on 18th May, 1937, and the third after the fifteenth exposure on 27th May, 1937, in malt-agar tubes. Complete recovery was noted in all these subcultures in the course of four days, conidia developed in large number and nowhere damage was found to persist. Wood-block *(Acacia)* subcultures were taken from the main plate and from the second subculture of the first generation on 28th May, 1937. The wood-block culture from the main plate represents the first generation while that from the second subculture represents the second generation. The first smear taken from both the wood-blocks on 4th June, 1937, *i.e.*, seven days after inoculation, showed complete recovery to the normal state. Growth was very vigorous in both. No fruiting appeared anywhere as it was a non-fruiting strain.

Influence of incandescent light (150 c.p. bulb)

(A) On culture of *Polystictus leoninus*.

A petri-dish culture of *P. leoninus* inoculated on 10th August, 1936, was exposed to incandescent light from 15th August (*i.e.*, five days after inoculation) to 5th September, 1936. During this period altogether sixteen exposures were given, each exposure being of 6 to 7 hours duration. The culture was placed at a distance of 50 cm. directly below the source of illumination. The temperature recorded near the culture varied from 30° to 32° C. and this was always found to be higher by only one degree than the room temperature. The petri-dish lid was never replaced by cellophane so that a portion of the light was cut off by the glass. Exposures were always given in a dark room thus excluding all chances of outside illumination.

Results in the main plate

The hyphae were all binucleated with, as usual, a good number of mediate branches and a few chlamydospores before exposure was begun. There were no conidia and no trace of basidia.

(1) *Damage to the vegetative hyphae* by such long exposures to strong illumination was not much. The protoplasm of the vegetative hyphae gradually fragmented. A large number of hyphae were devoid of cell contents, lost their clamps and became converted into hairs. But this is quite a normal phenomenon and takes place in normal culture as it becomes old. No tendency to break up into oidia was noted. No conidia developed and the number of chlamydospores was never found to increase.

(2) *Damage to the reproductive stage.*

The first appearance of basidia was noted in smear only on 18th August, 1936, *i.e.,* 8 days after inoculation while the control fruited perfectly well after 6 days. The basidia were all immature, though present in good number. They were never found to be arranged in close cluster, but were always irregularly scattered. They never came to maturity and were never found to bear sterigmata or spores. The basidia, however, became much affected by radiation. They gradually became much shrunken in appearance, decreased in number and totally disappeared from the culture after the tenth exposure on 28th August, 1936. No fruiting area was formed upon the culture and no porous surface was formed, so that fruiting could not be detected from the appearance of the culture as a whole.

No further subculture or transfers to wood-blocks were carried out.

(B) On culture of *Polyporus ostreiformis.*

A petri-dish culture of *P. ostreiformis* inoculated on 10th August, 1936, was exposed in the same manner to incandescent light from 15th August, 1936, to 5th September, 1936. The details of the method were the same as in the previous experiment. Altogether sixteen exposures were given, each exposure lasting for 6 to 7 hours.

Results in the main plate

(1) *Damage to the vegetative hyphae* was slight. The hyphae were all binucleated, broad and narrow, with a good number of mediate branches when exposure was first begun. A few chlamydospores were present. This condition continued right upto the end but as the culture became old, the number of chlamydospores was found to increase and a good number of vegetative hyphae had protoplasm fragmented at places only. Some empty hyphae were also produced. But these are normal phenomena and cannot be attributed to the effect of strong illumination.

(2) *Damage to the reproductive phase* was very great. No fruiting ever occurred in the main plate while the control plate fruited perfectly well after 18 days. No moist spot developed in the main plate anywhere and the presence of basidia could not be detected even in smears.

No further subcultures or transfers to wood-blocks were carried out.

Influence of heat upon culture of *Trametes cingulata*

A full-grown plate of *T. cingulata* was exposed to heat. Half of the plate was placed over paraffin bath at 55° C. while the other half was projecting out and was held in position by clamp. The plate was heated for 5, 10, 20 and 30 minutes, but no damage was noticed either to the hyphae or to the conidia. Heating was then continued for 40 minutes. It was observed that some clamps had fallen out and that empty hyphae were found in fairly large number and conidia were killed in number. The control half was normal. The heating effect slowly passed away and the heated half gradually reverted to the normal state within two to three days, as revealed on smear-examination.

Another full-grown plate of *T. cingulata* was treated on the same day with X-rays at 150 K V., 3 m. a. at 30 cm. from the target for fifteen minutes. Half of the plate was exposed to X-rays while the other half which served as control was screened with lead rubber. On comparison damage was found to be greater with X-rays than with heat. Barnes (1) also held that X-rays alone were much more effective than increases of temperature.

Ultra-violet and Solar Radiations

Discussion and conclusion

Though in all three cases (*Polys. leoninus, T. cingulata* and *Polyp. ostreiformis*) there was finally reversion to the normal state within a varying period, it was clear from the foregoing results that increasing the number of exposures was more effective in causing damage both to the vegetative and reproductive phases than increasing the length of exposure, as held by Ramsey and Bailey (13). In the case of *Polyporus ostreiformis* the greatest damage to the reproductive phase (fruit-formation) was caused by ultra-violet rays of daily five minutes' exposures for fifteen days with the first vegetative generation subcultures, where no fruiting areas or pores or any basidia were found; whereas in the main plate irregular fruiting areas without pores and rudimentary basidia without spores were found. This might probably be due to the "latent period" or to the delay in the appearance of the effects of radiation in living organisms. In the case of *Polystictus leoninus,* however, the greatest amount of damage to the reproductive phase was caused in the main plate, where no porous surface and no erect fruit-body were formed though a few shrunken basidia were irregularly scattered without attached spores. In the first vegetative generation subcultures from the main plate exposed to daily radiation of five minutes for

fifteen days a porous surface was formed with basidia arranged in series. These basidia were without attached spores and in the final erect fruits the pore-tubes were empty. But in the first vegetative generation subcultures from the main plate, exposed to only twice fifteen minutes' exposures in a fortnight, erect fruits were formed with regular pores and basidia arranged in series with attached spores, but the sporefall in moist agar plate was scanty and spores were not viable. Normal viable spores from normal erect fruit-bodies were found only in the second generation subcultures where complete recovery was noticed.

The effect of exposure to direct sunlight minus ultra-violet rays but combined with higher temperature was much more pronounced than that of ultra-violet, X-rays or radium in both the vegetative and reproductive phases. The killing effect was evident after the second exposure and the cultures ultimately died, they could not be revived in subcultures; thus, the fruit formation was permanently checked. In the case of incandescent light (150 c.p. bulb) however, the damage to the vegetative hyphae was slight though in the reproductive phase the fruit-formation was ultimately suppressed. These Polypores are white without any pigment. Burkholder (4) has remarked that "where light exerts an action upon growth, it is probably brought about by absorbing substances (pigments) present in plants." Exposing culture of *Trametes cingulata* to heat (55° C.) for forty minutes some damage was produced, which however, passed off in the course of two to three days. Dickson (7) could get no saltation by application of heat. Comparison with X-rays shows that X-rays produce greater damage than heat alone. Ultra-violet rays had great killing effect on freshly shed spores on moist agar surface, with decrease of exposure to only five minutes some of the spores could germinate but they could not progress further. Stevens (17) also found that an exposure of ten minutes to ultra-violet rays killed the spores. My experimental results confirm the observations of Smith (16) that "the changes in fungi which have been described as due to the influence of visible and ultra-violet light have not been heritable. They have been in no sense mutations."

One common feature of these different modes of treatment was almost universal reduction in fertility, sometimes the action of the external agent was so violent as to cause the death of the organism or of the spore, that is, in such cases the damage has been so severe that it cannot be repaired. In other cases the damage has been of a temporary nature so that there is ultimate reversion to the normal form after a period of growth. Barnes (1) is probably right here in holding that treatment with violent external agents may well hasten the slow normal changes of a degenerative character, bringing about a general derangement of physiological balance of the cell. Karl Sax (14) holds that "heat and age seem to induce the same effects as X-ray treatment."

Influence of X-rays on artificial cultures of three Polypores

Two kinds of X-rays were employed—(1) hard rays from a Coolidge tube of 150 K. V. potential with tube-current of 3 milli amperes, the distance from the target being 30 cm. and (2) soft rays of 50 K. V. and 30 K V. potential with tube-current of 2 milli amperes and 3–5 m. a., the distance from the target being 18 and 10 cm. respectively.

Daily one hour (hard) X-ray exposure for 14 days at 150 K V., 3 m. a. on cultures of *Polystictus leoninus* and *Polyporus ostreiformis*.

Damage in the main plate

(1) A malt-agar plate of *Polys. leoninus* inoculated on 25th June, 1936, was taken. The culture received 14 exposures in all on almost consecutive days. With the exception of the first four exposures which were given for 30 minutes each, the remaining ten exposures were each of one hour's duration. Exposure was first begun on 1st July, 1936, *i.e.,* 5 days after inoculation and ended on 28th July, 1936. The petri-dish lid was replaced by cellophane (sterilised with alcohol) during exposure. The hyphae were with clamps and some mediate branches, they were non-conidial without any trace of basidia. A plate culture was kept as a control.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae The first exposure to x-rays produced very appreciable damage to the protoplasm and heavy damage was noticed after the fifth exposure. The changes produced in the vegetative hyphae were death and destruction, With increasing doses of radiation the protoplasm of living hyphae became more and more damaged and showed a very much vacuolated appearance or were in disconnected chains with a strong tendency to break up into oidia (i.e. conversion to the monocaryon stage). Clamps were destroyed in good many hyphae	Fruiting was greatly affected in the main plate. The first appearance of basidia was noted on 5th July (1936), i.e. 10 days after inoculation while the control plate produced perfect fruit on the 7th day after inoculation, so that fruit-formation was delayed by 3 days in the irradiated plate. The basidia at their first appearance were all in dense cluster but they did not bear sterigmata or spores, tramal hyphae bearing clamps but with no terminal spores were sometimes found amongst the basidia. Some abortive fruit-bodies appeared in the main plate after 15 days and these on sec-	No saltant hyphae were produced. Not only fruit-formation was delayed by 3 days, but the development of reproductive bodies was almost entirely suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
and numerous extremely narrow non-clamped hyphae in addition to mediate branches were produced. But the number of clamped hyphae (dicaryon ones) preponderated. Empty hyphae and hairs were produced in large number. (*b*) A small number of conidia appeared at the end on 28th July 1936, i.e. 34 days after inoculation. (*c*) Chlamydospores which at first were few, were found to increase in large numbers later on.	tioning several days after they had been formed, were found to consist only of hairs, not even any pores were formed. Basidia were scarcely visible on smear examination, they were few and irregularly scattered and very much shrunken.	

(2) A malt-agar plate was inoculated with *Polyporus ostreiformis* on 25th June, 1936, and was exposed to X-rays. The lid of the petri-dish was replaced by cellophane during exposure. Exposure was first given on 30th June, 1936, *i.e.*, 5 days after inoculation and ended on 27th July, 1936. During this period altogether 14 exposures were given. With the exception of the first four exposures which were given for 30 minutes each, all other exposures were of one hour's duration. The hyphae were all broad with clamps, measuring 4–6μ in breadth, with a good number of mediate branches and chlamydospores, they were non-conidial when the exposure was begun. A plate-culture was kept as a control.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Damage to the vegetative hyphae was first noticed after the third exposure on 3-7-36, the first and the second exposures having no effect. The damage was very slight, the	No fruiting ever occurred in the irradiated plate, though the control plate fruited perfectly well on 14-7-36 (after 19 days). A moist spot developed on the wall of the irradiated plate on 18-7-36 (23 days) but this did	Fruit-formation was entirely suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
protoplasm of living hyphae having become broken up at places only. With increasing exposures, the damage became more and more pronounced. Vacuolation went on increasing resulting in the breaking up of the continuous mass of protoplasm into disconnected masses. In many hyphae, the protoplasm contracted from the cell-wall and presented a streaked appearance, showing a tendency to breaking up into oidia. A large number of hyphae became dead and empty and many such empty hyphae with clamps partially or totally empty but still attached, could be found. Narrowing of hyphae was another feature which became evident after increasing exposures and broad healthy hyphae totally disappeared. (*b*) A few conidia developed after the 13th exposure, i.e. 28 days after inoculation. But their number never increased. (*c*) The number of chlamydospores became more and more reduced till they disappeared entirely at the end.	not develop any further and did not show any differentiation into porous area. A smear examination from this moist spot showed a good number of hyphae with swollen club-shaped ends suggestive of rudimentary basidia.	

Changes in sub-cultures from the main plate (*i.e.*, in the first vegetative generation)

(1) In the case of *Polystictus leoninus* altogether six subcultures were carried out from the main plate; the first after the first X-ray exposure on 1st July, 1936, and the second, after the third exposure

on 3rd July, 1936. The rest became contaminated and were, therefore, rejected. The smear-examination of these two subcultures gave almost identical results.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae The protoplasm of vegetative hyphae presented a much damaged outlook and was much vacuolated or in disconnected chains. The hyphae became all narrower than the normal, and extremely narrow hyphae could be found. Empty hyphae without clamps and living hyphae with totally or partially empty clamps were found in large number and all of them became ultimately converted into dead double-walled hairs. But always clamped hyphae preponderated over the non-clamped ones. (*b*) Conidia in non-clamped hyphae appeared in the first sub-culture after 27 days and in the second sub-culture after 25 days in large numbers. (*c*) Chlamydospores appeared in large numbers in both the first and second sub-cultures.	Irregular fruit-bodies appeared in both the subcultures after ten days as could be seen from the appearance of toothed areas round the peripheral regions of the cultures, the control fruited on the eighth day. Basidia developed in large number at first but they were always irregularly scattered among masses of dead double walled hairs with neither sterigmata nor spores; a few tramal hyphae with only clamps were intermixed with them. The basidia were of irregular shape and appeared very much shrunken even at their first appearance. As the cultures grew older, the basidia disintegrated and disappeared.	As in the main plate not only fruiting was delayed but the formation of regular perfect sporophores was altogether suppressed.

(2) In the case of *Polyporus ostreiformis* altogether five subcultures were carried out from the main plate; the first after the first exposure, the second after the third exposure, the third after the sixth exposure, the fourth after the eighth exposure, and the fifth after the fourteenth or the last exposure. All these subcultures were in malt-agar plates.

Damage

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. All subcultures showed the damaged character of the vegetative hyphae, but the amount of damage was far greater in the last two subcultures than in others. (*b*) No conidia or any dead hyphae were found. (*c*) Chlamydospores were present in all in fairly good number.	No fruiting occurred in any plate but hyphae with swollen ends suggestive of rudimentary basidia could be found intermixed with damaged hyphae in all plate-cultures.	As in the main plate the reproductive phase was totally checked. No moist spot ever developed on the wall of the plates.

Changes in the second set of sub-cultures from the first set (*i.e.*, in the second vegetative generation)

(1) In the case of *Polystictus leoninus* two subcultures from the main plate were again subcultured in plates on 5th August, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Very few non-clamped hyphae were present and the condition of the protoplasm resembled normal. (*b*) Conidia were produced in moderately good number. (*c*) Chlamydospores were also found in good number.	Fruit-body appeared in both these sub-cultures after eight days (*i.e.*, 13th August, 1936) and this was the fruiting period for the normal at that time. Toothed areas showing regular pores developed and a section showed a few basidia intermixed with a few tramal hyphae bearing clamps.	It is thus found that with the exception of suppression of spore-formation there was definite reversion of the vegetative hyphae to the normal condition, and possibly complete reversion would have occurred if the plates were again subcultured as soon as they were filled up.

(2) In the case of *Polyporus ostreiformis,* the last two subcultures showed a greater amount of damage than others. They were again sub-cultured in malt-agar tubes on 1st August, 1936. A smear-examination on 4th August, 1936, showed complete recovery of damaged hyphae to the normal state. But no fruit-body appeared in them as they were in tubes and not in plates, no fruit-body having occurred at that time in tubes even in controls.

Recovery from damage

(1) In the case of *Polystictus leoninus* damage appeared in the main plate after the first exposure on 1st July, 1936. Recovery of the vegetative hyphae was noted in both the subcultures of the second vegetative generation on 10th August, 1936, *i.e.*, after 39 days.

Fruit-body appeared		Control
In Main plate	in 10 days	Fruited in 7 days
In Subcultures :		
(*a*) 1st. generation	,, 10 days	,, ,,
(*b*) 2nd. generation	,, 8 days	,, 8 days

(2) In the case of *Polyporus ostreiformis*, damage to the vegetative hyphae occurred first after the third exposure on 3rd July, 1936. Recovery of the vegetative hyphae was noticed first in tube subcultures of the second vegetative generation on 4th August, 1936, *i.e.*, after 32 days.

Daily 15 minutes (hard) X-ray exposure for seven days at 150 K V. with three milli amperes on cultures of *Polystictus leoninus* and *Trametes cingulata*

(1) A malt-agar plate was inoculated with *Polystictus leoninus* on 9th June, 1936. The culture was exposed to X-rays from a Coolidge tube at 150 K V. 3 m. a. for seven days for fifteen minutes on each day. The first exposure was given on 13th June, *i.e.*, 4 days after inoculation, and the last, on 22nd June, 1936. The petri-dish lid was replaced as before by cellophane sterilised with alcohol. There were no basidia when exposure was begun, the strain showed hyphae with clamps and with a number of mediate branches and there were no conidia or chlamydospores or dead double-walled hairs. A separate malt-agar plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. The changes in the vegetative hyphæ were that they became more and more vacuolated, and with increasing	The first appearance of basidia was noted on the eighth day after inoculation (i.e. on 16th June) which falls within the normal period. Regularly	The chief peculiarity of the damage was that mature spores were never found on the sterigmata and the basidia were of an irregular type like an

In the vegetative stage.	In the reproductive stage.	Observations.
exposures they lost their clamps and protoplasmic contents and were ultimately converted into dead double-walled hairs. Non-clamped hyphae with protoplasm broken up into pieces were produced in good numbers but they were only stages in the formation of hairs. No narrow hyphae were produced. It was found on the whole that clamped hyphae preponderated over the non-clamped ones. This type of changes was also noticed when a normal culture becomes old, thus the damage produced was not very significant. (*b*) No conidia were produced. (*c*) A few chlamydospores appeared after the last exposure but their number never increased.	raised and toothed areas were formed in concentric zone half way between the inoculum and the edge of the culture, and erect fruit bodies showing regular pore-tubes appeared here and there on this zone after an interval of eleven days from the date of inoculation. The pore tubes showed a well developed hymnium consisting of basidia with sterigmata and attached immature spores, mixed with a number of cystidia and tramal hyphae bearing clamps but no terminal spores.	inverted flask, very few being clavate. Thus, the fruit-formation was partially affected.

Damage in sub-cultures

A subculture was carried out in plate after the fifth exposure on 19th June, 1936. Smear-examinations from this subculture were identical in all respects to those from the main plate. Fruit-formation occurred after 10 days and was delayed by 3 days in comparison with the control. Pore-tubes were well-developed but they were all empty showing no trace of basidia or other organs and filled with dead tramal hyphae. The effects of radiation are, therefore, clearly evident in the subculture, though not to the same degree in the main plate, as there was total suppression of the development of reproductive organs.

On 25th June, 1936, a wood-block (mango-wood) subculture was carried out; growth on this wood-block culture was fair but it did not ultimately produce any fruit-body.

(2) A malt-agar plate was inoculated with *Trametes cingulata* on 9th June, 1936, and was exposed in the same manner to X-rays

at 150 K V., 3 m. a., at 30 cm. from the target for seven days for fifteen minutes on each day. Exposure was first given on 13th June, 1936, *i.e.*, 4 days after inoculation and ended on 20th June, 1936.

Damage in the main plate

The only effect produced was that living hyphae became dead and empty of cell-contents in large number. Such dead hyphae increased with increasing doses of X-rays. Living hyphae were all with clamps and with numerous mediate branches. Conidia became enormous with a good number of chlamydospores.

Recovery from damage in sub-cultures

Three subcultures were taken, *viz.*, after the first, third, and the seventh exposures. They showed no dead or damaged hyphae and were absolutely like the normal in every respect.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 23rd June, 1936. Growth was rather stunted and checked at first, but later on the wood became completely covered up. No fruit-formation occurred in wood-block, in the irradiated plate or in any subculture.

The effect of X-ray exposure for *only fifteen minutes* on culture of *Trametes cingulata* at 150 K V. with 3 m. a. at 30 cm. distance from the target was studied. A malt-agar plate was inoculated with *Trametes cingulata* on 30th May, 1936. It was exposed to X-rays from a Coolidge tube only once on 6th June, 1936, (when the plate was full), *i.e.*, 7 days after inoculation, for a period of fifteen minutes only. The petri-dish lid was replaced by cellophane during exposure. The hyphae were all with clamps and with numerous mediate branches and conidia at the time of exposure and the culture showed distinct zonation. There were no chlamydospores.

The only effect produced by fifteen minutes' radiation was that a number of hyphae became dead, and many were damaged and lost clamps. Chlamydospores developed later as in the normal. Smear-examination from the zoned area showed numerous conidia and mediate branches while that from non-zoned area showed less conidia and less mediate branches. These features are also present in a normal culture showing zonation.

A mycelial transfer was made to sterilised wood-block (mango-wood) on 15th June, 1936. Moderately good growth was produced and smear-examination showed normal type of hyphae with numerous mediate branches, conidia and a few chlamydospores. No fruit-body appeared either in the irradiated plate or on the wood block.

Daily one hour X-ray (soft) exposure upon *Polystictus leoninus* for six days at 50 K V. 2 m. a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 13th November, 1936, was exposed to X-rays. The first exposure was given on 16th November, *i.e.*, 3 days after inoculation, and the last on 26th November, 1936. Altogether six exposures were given within this period and each exposure was of one hour's duration. The petri-dish lid was, as usual, replaced by cellophane. The potential and tube current employed in the case were 50 K V. and 2 m. a. respectively and the distance of the plate culture from the target was 18 cm. The hyphae at the start were all broad, with clamps and with a good number of mediate branches. There were no conidia, but a few chlamydospores were present and there was no trace of basidia when exposure was first begun. A separate plate-culture was kept as control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Damage to the vegetative hyphae appeared first after the second exposure on 18th November, 1936, the first exposure having no effect. Hyphae became a little narrower than the normal and their protoplasmic continuity became broken by at places only. With increasing doses of radiation many more hyphae became more and more damaged and narrow. Vacuolation increased and the whole protoplasmic mass of living hyphae became broken up into disconnected chains. Some extremely narrow damaged hyphae without any clamps were produced. But their number always remained fewer and clamped hyphae whether broad or narrow, always preponderated over non-clamped ones. A good number of empty hyphae were produced and	Basidia first appeared in the irradiated plate on 20th November,'36, so that fruit body appeared after seven days. The control plate also fruited at the same time. But contrasted with the control, fruiting was very much affected, no regular and erect fruits developed on the culture. Some yellow porous areas were formed but these were distributed in patches over the culture and were not aggregated to form a complete circular zone round the inoculum. Smear-examination from these fruiting areas showed an immense number of dead hairs amongst which were found a few basidia The basidia were much smaller in size than those of the normal, were much shrunken in appearance and irregular in shape and were never in close cluster but irregularly	Basidia were found in good number when they first appeared, but with increasing exposures their numbers became much reduced. This was due to a large number of basidia undergoing disintegration and this was especially evident after the fifth exposure when a large number of disorganised basidia became aggregated in masses, took a deep stain and lost their distinct outline. No fruit-body was inserted in agar plate for spore discharge as no regular fruit could be found.

In the vegetative stage.	In the reproductive stage.	Observations.
these became double walled and were ultimately converted into hairs which increased in immensed number. (*b*) A few conidia were produced after an interval of twenty-one days after inoculation. (*c*) Chlamydospores entirely disappeared.	scattered, and they were never found with sterigmata or attached spores.	

Changes in the first vegetative generation-Sub-cultures

Altogether five subcultures were carried out from the irradiated plate, the first after the first exposure, the second after the second exposure, the third after the fourth exposure, the fourth after the fourth and last exposure, and the fifth, 7 days after the last exposure. The first four subcultures were in plates while the last was in trube.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. While the first and the second subcultures showed hyphae which were all extremely narrow in comparison with the normal, with clamps extremely reduced in size wherever present, and with an abundance of such hyphae rendered clampless by radiation and with protoplasm invariably broken up in disconnected chains, the third and the fourth subcultures showed hyphae of similar nature together with hyphae almost resembling normal condition in about equal proportions; the fifth sub-culture showed an overwhelming preponderance of normal hyphae.	Fruit body appeared in all after eight days which falls almost within the normal period. The fruit-bodies were all very regular in appearance, showing well-formed poretubes, and a fruit from each sub-culture was inserted in agar plate on 16th December 1936. No spore-fall occurred from the fruits of the first four sub-cultues but spores were discharged from the fruit of the fifth subculture twice on 17th December, 1936 and 18th December 1936 though very scanty each time. Sections were taken from all and it was found that while the fruits of the first four sub-cultures had pore tubes filled only with dead tramal	In general, the damage caused in the main plate by radiation was found to persist in all but the degree of damage present varied to a considerable extent in each sub-culture. The first and the second sub-cultures showed damage to the same extent as that of the main plate, the third and the fourth sub-cultures showed damage to a far lesser extent, while the fifth sub-culture showed only slight damage when compared with the normal.

In the vegetative stage.	In the reproductive stage.	Observations.
(*b*) Conidia whose presence in a non-conidial strain like *P. leoninus* is always associated with damage, appeared in all the subcultures within 6 to 8 days after inoculation. Their number was appreciably great in the first and second sub-cultures but became a few only in the third and fourth sub-cultures. The fifth sub-cultures whose condition was almost similar to the normal, had almost no conidia but a few chlamydospores. (*c*) No chlamydospores were found in the first four sub-cultures, a few chlamydospores were present in the fifth sub-culture.	hyphae, that of the last had a few living tramal hyphae provided with clamps but no terminal spores. Probably, the few basidia that shed spores had all been converted into tramal hyphae by the time the fruit of the last sub-culture had been sectioned. The spores were transferred to a malt-agar tube and kept inside an incubator at 37° C. Germination occurred after an interval of nine days from the date of transfer and clamps developed after eleven days. The tube was brought to light and a small yellow patch on the top of the plant suggesting a normal sporophore developed after sixteen days.	

Results in the second vegetative generation-Sub-cultures

The five subcultures of the first generation were transferred to fresh malt-agar tubes, the first and the second on 2nd December, 1936 and the rest on 5th December, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Smear-examination on 9th. December, 1936 showed that in every tube the majority of vegetative hyphae had reverted to the normal stage. Damaged hyphae showing broken up nature of protoplasm still persisted in small number but they	Fruit-body appeared in all the five tubes within the normal period after seven days. Basidia in close cluster, some even with sterigmata and attached spores, could be found on smear-examination. The fruit-bodies were all well developed showing regular poretubes and	Smear-examination showed absolutely normal hyphae of *P. leoninus* with clamps and mediate branches. Thus, complete recovery was obtained in the second vegetative generation.

In the vegetative stage.	In the reproductive stage.	Observations.
gradually disappeared from the cultures later on and normal healthy hyphae came into preponderance. (*b*) A small number of conidia developed in all but their number never increased. (*c*) Chlamydospores developed in all and they increased to good number, later on.	were inserted in agar plate on 21st December, 1936. No spore-fall occurred from the fruit of the first sub-culture (probably due to some defect in the mode of insertion) but spore-fall was obtained from all others on 23rd December, 1936 and continued for two days, though very scanty on each day. Sections showed that the poretubes contained basidia mixed with a few tramal hyphae bearing clamps but no terminal spores. But no attached basidiospores were found. The basidia were all of normal size and measured 26x10 μ. Spores from each of the four fruit bodies were then transferred to malt-agar tubes and kept inside an incubator at 37° C owing to the low temperature-conditions of the time. The spores germinated after five days and imperfect fruit-bodies appeared after twelve days (owing to being kept in the dark).	

Further sub-cultures

Mycelia from the above five tubes of the second vegetative generation were subcultured in malt-agar tubes upto the fifth generation but in no case was damage found to persist. Fruit-body appeared in every generation within the normal period and perfect and regular fruit-bodies were produced. The fruit-bodies were employed for various purposes when fresh spores were required, and in every case copious spore-fall was obtained from fruit-body of each tube. In every generation chlamydospores were totally absent when the culture was young and ultimately became numerous when the culture turned old. Conidia developed in very small number in every generation. They never increased in number and persisted right upto the end.

Growth in wood-blocks

Inocula from the main plate as well as from the first two subcultures of the first generation which showed the greatest amount of damage, were carried to sterilised wood-blocks (mango) on 30th November, 1936. Growth was moderately good in all. The wood-block inoculated from the main plate showed recovery on 22nd February, 1937, *i.e.*, after 85 days, while those inoculated from the two subcultures showed recovery after 50 days on 18th January, 1937. Fruit-body appeared in the wood-block subculture from the main plate on 13th April, 1937, *i.e.*, 104 days after inoculation and 19 days after recovery, while fruit-bodies in the other two wood-blocks occurred on 8th March, 1937, *i.e.*, 68 days after inoculation and 18 days after recovery. Control wood inoculated in April, fruited after 14 days. A small part of the sporophore from each of the three wood-blocks was taken and inserted in agar plates on 16th April, 1937. Spore-fall occurred from each of the two fruit-bodies of the wood-block subcultures from two first generation subcultures, though it was rather scanty. Spores germinated quickly inside a moist belljar. But the fruit-body from the wood-block subculture from the main plate did not shed any spores, though section showed that the pore tubes were densely lined with basidia measuring 26–30 $\times$ 10μ mixed with a good number of tramal hyphae bearing clamps only. No sterigmata or attached spores were found.

Recovery from damage

Damage appeared in the main plate of *Polystictus leoninus* after the second exposure on 18th November, 1936, and recovery of vegetative hyphae was noted in tube subcultures of the second vegetative generation on 9th December, 1936. The total period calculated from the date of damage was therefore 22 days.

Fruiting period of		Control fruited in	
(*a*) The Main plate	7 days	7 days	
(*b*) Sub-cultures :—			
(1) 1st. generation	8 days	6-8 days	
(2) 2nd. generation	7 days	7 days	
(3) 3rd. generation	10 days	10 days	winter months
(4) 4th. generation	11 days	11 days	
(5) 5th. generation	10 days	10 days	

Fruiting period in wood-blocks

	Total period	Period after recovery	Fruiting period in the control wood-block
Subcultures from the main plate	104 days	19 days	14 days
,, 1st. subculture	68 days	18 days	
,, 2nd. ,,	68 days	18 days	

16 hours' (soft) X-ray exposure upon *Polystictus leoninus* at 30 K V. 3-5 m.a.

A malt-agar plate-culture of *Polys. leoninus* inoculated on 5th February, 1937, was exposed to X-rays on 11th February, 1937, from a Heading tube which enabled exposures to be given for hours together without interruption. The potential was only 30 K V. and the tube-current varied from 3 to 5 m. a. By increasing the tube-current and lowering the potential, not only the output of X-rays increased but also the rays became very superficial—though much more penetrating than the ultra-violet rays. The lid of the petri-dish containing the culture was replaced by sterilised cellophane during the exposure, and the plate was fixed in front of one of the windows of the apparatus by clamps in such a manner that the rays fell vertically upon the culture, affecting an area of about 3 cm. in diameter round the inoculum. The distance of the culture from the target was kept at 10 cm. Exposures were given for five hours on 11th February, 1937, five hours on 12th February, and for six hours on 13th February, 1937, so that the total period of exposure was sixteen hours.

As the rays affected only a small area round the inoculum, two subcultures were carried in petri-dishes, immediately after the third day's exposure—one of this was from the irradiated region and the other from the non-irradiated region near the margin of the exposed plate. Another subculture was taken in malt-agar tube from the irradiated region six days after the last exposure. The irradiated plate and the subcultures were all kept in the diffused light of the room.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Smears were taken from both the irradiated and non-irradiated	Fruit-body appeared in the main plate after thirteen days while the control fruited after nine days. Excepting	The damage that was produced by three exposures of total sixteen hours' duration was almost insignificant and

In the vegetative stage.	In the reproductive stage.	Observations.
regions of the culture. While smears from the non-irradiated region were absolutely like those from the normal, smear-examination from the irradiated region showed hyphae with prtoplasm slightly damaged and broken up at places only and clamps a little smaller than those in the normal. (*b*) Conidia developed eight days after inoculation and were found when the culture was first examined after the third exposure. But conidia were always a few in number and were restricted only to the irradiated portion. (*c*) Chlamydospores developed eight days after inoculation and were found when the culture was first examined after the third exposure, subsequently chlamydospores increased in large number and were found throughout the culture.	a delay of four days in the fruiting period no other effect was produced. Sporophore appeared normally in a circular zone round the inoculum and regular pores were formed. Smear-examination from these fruiting areas showed basidia in large number arranged in compact layer with sterigmata and attached spores, mixed with a large number of tramal hyphae bearing clamps but no distinct secondary spores. Basidia and spores were all healthy and agreed with the normal in measurements.	indistinguishable from the normal state.

Results in the sub-cultures

The two subcultures after the last exposure from the irradiated and non-irradiated portions in malt-agar plates fruited after twelve days, while the subculture in a malt-agar tube after an interval of six days from the last exposure fruited after nine days. Fruiting was, therefore, delayed by three days in the first two subcultures but occurred within the normal period in the case of the third subculture. On smear-examination, however, they were all found to be absolutely normal in the vegetative character of the hyphae and no damage was found in any of them. Fruit-bodies were very regular in all of them, showing basidia with attached spores, tramal hyphae, etc. Thus, except a retardation of three days in the appearance of fruit-bodies in the first two subcultures no other effect was produced.

Irradiation of spores by soft X-rays

Fresh spore-fall from a normal fruit-body of *Polystictus leoninus* in tube was obtained on two agar plates on 16th March, 1937. One plate was immediately exposed to soft X-rays at 50 K V., 2 m. a., from a Coolidge tube at a distance of 30 cm. from the target. Exposure was given for fifteen minutes and the petri-dish lid was replaced by sterilised cellophane during the exposure. Immediately after the exposure the irradiated plate as well as the other plate, which was kept as a control, were both kept inside an incubator at 37° C.

On 17th March, 1937, it was found that with the exception of a few spores all the irradiated spores had germinated. The control spores also germinated on 17th March, 1937. Subcultures were at once carried out in malt-agar tubes both from the control and irradiated spores. The tubes were kept inside incubator (in the dark). No growth occurred in any tube within five days, but on 23rd March 1937, further growth was noticed in all tubes containing irradiated as well as non-irradiated spores. Initiation of fruiting occurred in all tubes after seven days, but it took seven days more to develop tiny fruit-bodies showing regular pores as all the tubes were kept in the dark. Hyphae of subcultures obtained from X-rayed spores were similar in all respects to the normal ones, and no damaged hyphae were noticed. Two such fruit-bodies from irradiated spore-subcultures together with two control fruits were inserted in agar plates on 9th April, 1937. Copious spore-fall occurred from all the fruit-bodies within a few hours of insertion and continued for two days. Transfers of spores were again made to malt-agar tubes on 11th April, 1937, and fruiting occurred in all tubes within seven days. This time the tubes were kept in light. Sections showed preponderance of basidia in almost every pore-tube, bearing sterigmata and attached spores, mixed with a few tramal hyphae bearing clamps but no terminal spores. The basidia were $26–30 \times 10–12\mu$ and spores were hyaline, cylindrical and $10–12 \times 5–6\mu$. This agrees exactly with the normal in measurements.

X-RAYS

Discussion and conclusion

The only work on effects of X-rays on Polypores so far attempted, I think, is that of Dickson (7). He irradiated young cultures of *Trametes serialis* and *Merulius lachrymans* in petri-dishes but could not get any positive result, the only change noticed was a slowing up of the growth rate due to the heating effect during irradiation. In the present case also only negative result was obtained, no saltant or mutant involving genic change was ever produced. With lower fungi (Mucor, Phycomyces and Chaetomium), however, Nadson and Philippov (12) and Dickson (7 & 8) obtained saltants which remained constant through a number of succeeding

generations. As with the ultra-violet radiation various degrees of injury were produced by X-rays, which have been recorded in the preceding pages. From the application of two kinds of X-rays—hard one of 150 K V. and soft rays of 50 K V. and 30 K V. it was found that small doses produced slight injuries from which there was quicker recovery, and that heavy doses totally suppressed sporophore-formation though conidia and chlamydospores (asexual spores) remained almost unaffected. Nadson and Philippov (loc. cit.) could destroy the formation of sexual organs (zygotes) of *Mucor* by strong doses of X-rays but they could never prevent the formation of asexual sporangia. Smith (16) also holds that "fungi are rather insensitive to X-rays but large doses produce killing effects." Killing effects are caused probably by toxic substances in the cytoplasm of cells exposed to the action of X-rays. In connexion with his study of disintegration of chromosomes at the first meiotic division in three species of *Orthoptera* by application of X-rays Whito (18) holds that their complete disintegration results from the destructive action of a substance produced or liberated in the cytoplasm as a result of irradiation.

Effects of radium-radiation on artificial cultures of three *Polypores* (*Polystictus leoninus, Trametes cingulata* and Polypores *ostreiformis*)

As in the case of X-rays both small and heavy doses were tried —5 mg. radium in the case of light dose and 120 mg. radium as heavy dose. The radium used was in the form of radium sulphate enclosed within small platinum capsules ·1 mm. thick, which transmit mostly Y-rays. In each case the radium was placed on the upper surface of the lid of the culture-dish at the centre. In a malt-agar plate irradiated with 15 mg. radium for half a minute and then inoculated with *Polyporus zonalis* and *Trametes cingulata* it was found that the growth was normal as in a non-irradiated plate.

Light dose of 5 mg. radium

(1) Two malt-agar plate-cultures of *Polystictus leoninus* inoculated on 21st July, 1936, were exposed to 5 mg. radium rays for one minute and half a minute on 24th July, *i.e.,* three days after inoculation. Subsequent smear-examination showed no damage to the vegetative hyphae and fruiting areas were formed in both the plates one day earlier than that in the control plate. Excepting a slight hastening of the reproductive period, the effect of such short exposures can neither be regarded as exerting any stimulating influence. Subcultures were carried up to the second vegetative generation, the growth was found to be perfectly normal and fruit-formation was regular as in the normal cultures.

(2) A malt-agar plate-culture of *Trametes cingulata* was subjected to 5 mg. radium rays for one minute on the eighth day from the inoculation date when the plate was completely full and showed distinct zonation in the culture. Hyphae were with clamps and a number of short mediate branches, it was a conidial strain full of

conidia, such highly conidial strains usually do not form fructifying areas. The plate was microscopically examined every day for more than a fortnight, but during the period absolutely no change was found. Subsequent subculture on sterilised wood-block showed normal vigorous hyphae with a number of conidia and chlamydospores.

(3) Another similar plate-culture of *Trametes cingulata* was similarly exposed to 5 mg. radium rays for five minutes. A good number of hyphae became damaged by radiation, the protoplasm of such hyphae became broken up into disconnected chains of oidia and many became altogether empty, and there was great increase in the number of conidia and chlamydospores. But subsequent transfers to sterilised wood-blocks showed almost complete recovery in the course of about three months.

(4) A half-grown malt-agar plate-culture of *Trametes cingulata* inoculated on 13th June, 1936, was exposed to 5 mg. radium rays for six hours on 17th June, 1936, *i.e.,* four days after inoculation. Smears were examined at every hour and a subculture was taken at the end of each hour of exposure.

Results in the main plate

The culture was all with clamped hyphae and with a large number of mediate branches, a few conidia and no chlamydospores were noticed before the exposure was begun. During the continuance of exposure a few dead and empty hyphae were produced and there was a progressive increase in the number of conidia at the end of each hour. No chlamydospores were produced. Smear-examination on the next day *i.e.,* 18th June, 1936, showed the presence of quite a good number of dead and empty hyphae. The protoplasm of living hyphae became broken up into fragments at many places and presented a damaged appearance. A slight increase in the number of mediate branches was noted. Conidia increased in large number and a good number of chlamydospores was produced.

Results in the plate sub-cultures

Altogether six sub-cultures were carried out on the 17th June 1936, one at the end of each hour's exposure. Complete recovery to the normal state occurred in all the subcultures on the 23rd June, 1936.

A transfer was made to sterilised wood-block (mango) on 23rd June, 1936. Smear-examination after a month showed a preponderance of conidia over chlamydospores. The majority of the hyphae were as broad as normal hyphae and were in the living condition, but a few dead hyphae also were present.

(5) A malt-agar plate-culture of *Polystictus leoninus* also was similarly exposed to 5 mg. radium rays for six hours continually on 17th June, 1936, after an interval of four days from the inoculation date. As in the case of *Trametes cingulata* smears were examined

at every hour and a subculture was taken at the end of each hour of exposure.

Results in the main plate

During the continuance of exposure no damage was caused to the vegetative hyphae, no conidia were produced, chlamydospores which were a few before the exposure was begun, remained the same. Subsequent examination of the irradiated plate did not show any damage to the hyphae, only the chlamydospores increased in fairly large number but there was never any conidia produced nor did the hyphae show any tendency to break up into oidial chains. Fruit-formation was not affected in any way, fruiting areas were formed within the normal period (seven days) and they showed regular and healthy basidia and attached normal spores.

Results in the sub-cultures

Examination of the six subcultures also showed no damage anywhere, erect fruiting areas were formed within the normal period and they were perfect with normal basidia in close clusters mixed with a few tramal hyphae clamped, and some of the basidia had sterigmata bearing mature spores.

Heavy dose of 120 mg. Radium

(1) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 25th June, 1936, was exposed after four days, *i.e.,* on 29th June, 1936, for 48 hours continually to 120 mg. of radium. The petri-dish containing the culture was kept inverted during exposure and the radium was placed upon the upper surface of the bottom part of the petri-dish. The hyphae were all with clamps and with a good number of mediate branches. The culture was absolutely young, showing no conidia or chlamydospores or hairs and there was no trace of basidia when the exposure was begun. A separate plate-culture was kept as a control. Subcultures from the main plate were taken after the removal of the radium (after 48 hours).

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damage to the vegetative hyphae became evident on the very first smear-examination on 2nd July, 1936. The protoplasm of living hyphae became much vacuolated and broken up into fragments. As the culture became old, these damaged hyphae gradu-	Basidia first appeared in the main plate on 5th July, 1936 *i.e.,* 10 days after inoculation while the control fruited perfectly well after seven days. Fruit-formation was characterised by the appearance of toothed areas round the periphery of the culture. Smears from this area showed	The results of irradiation in the main plate are therefore in the direction of suppression of spore-formation, delayed and imperfect development of fruit-bodies and a gradual conversion of healthy and clamped hyphae into non-clamped damaged ones.

In the vegetative stage.	In the reproductive stage.	Observations.
ally increased in number and healthy hyphae almost disappeared. The protoplasm became more and more vacuolar and ultimately broke up into disconnected chains with a strong tendency to break up into oidia. Many of these hyphae altogether lost their cell contents and clamps and were ultimately converted into dead and double-walled hairs. Furthermore, the damaged hyphae became much narrower than the normal ones, clamps became extremely reduced in size, and the protoplasm presented a very much streaked appearance. But the number of clamped hyphae always preponderated over the non-clamped damaged hyphae. The number of dead hairs and empty hyphae gradually increased.	basidia in large number for the first few days. But these basidia never came to maturity. They were never found to bear any sterigmata or spores, were very much shrunken on the first appearance and were either irregularly scattered or sparsely clustered. A few living tramal hyphae could occasionally be found but no clamps or terminal spores were found in them. An erect fruit showing regular pores developed, however, on the culture on 10th July, 1936, *i.e.*, after fifteen days, but on sectioning it was found that the pore-tubes were filled only with dead tramal hyphae and there was no trace of any living element. The basidia that were found in smears for the first few days gradually disintegrated later on.	
(*b*) and (*c*) Conidia and Chlamydospores.		
Conidia appeared almost simultaneously with chlamydospores on 27th July, 1936, *i.e.*, 32 days after inoculation and both could be found in large numbers later on. But the number of chlamydospores was always greater than that of the conidia.		

Damage in the sub-cultures (*i.e.*, first vegetative generation)

Two sub-cultures were carried out from the main plate, the first on 6th July, 1936, *i.e.*, 5 days after the removal of radium, and the second on 8th July, *i.e.*, 7 days after its removal.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damage caused in the main plate by radiation was fully transmitted to the sub-cultures. The vegetative hyphae were all extremely narrower than the normal with protoplasm broken up into disconnected chains of oidia. Healthy hyphae were altogether absent. A large number of such hyphae became non-clamped, emptied of all cell-contents and were converted into hairs. Clamps became extremely reduced in size and the number of such damaged hyphae with extremely reduced clamps were almost equal to those which became non-clamped. (*b*) Conidia developed in small number simultaneously with chlamydospores. Conidia were first noticed in the first sub-culture on 23rd July, 1936, *i.e.*, seventeen days after inoculation, and in the second subculture on 20th June, 1936, *i.e.*, 12 days after inoculation. (*c*) Chalamydospores and conidia both increased in large numbers later on but the number of conidia was found to be preponderating over that of the chlamydospores.	Fruiting areas as noted by the first appearance of basidia, appeared in the first sub-culture on 14th July, 1936, *i.e.*, eight days after inoculation and in the second subculture on 15th July, 1936, *i.e.*, seven days after inoculation. The fruiting period for the control at that time was seven days. Toothed areas developed round the periphery of the cultures and smear-examination from these areas showed a large number of basidia irregularly scattered and not in cluster. Basidia were much shrunken in appearance and without any sterigmata or spores. Erect fruit-bodies developed in both the plates, but on sectioning they did not show any basidia in the pore-tubes but a few living tramal hyphae bearing only clamps and no secondary spores.	No further transfers were made to any wood-block and the recovery process could not be studied owing to accidental contamination of both the sub-cultures in the first generation.

(2) A malt-agar plate-culture of *Polyporus ostreiformis* inoculated on 25th June, 1936, was subjected to 120 mg. radium on 29th June, 1936, *i.e.*, four days after inoculation when the plate was almost full. Radium was placed upon the upper glass surface of

the lid of the petri-dish and removed after 48 hours on 1st July, 1936. The plate-culture showed microscopically all broad and clamped hyphae measuring 4–6μ in breadth with a good number of mediate branches. The clamps were all broad measuring 3–4μ in breadth. There were no conidia but a good number of chlamydospores were present. A separate plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Damage to the vegetative hyphae was not very great. The first sign of damage was noted on 6th July, 1936, *i.e.*, five days after removal of the radium. The protoplasm of some hyphae showed an increased vacuolation and became streaked in appearance or broke up at places only. A few dead and empty hyphae without any clamps were produced and these increased to good number later on. Excepting these changes no other effect was noticed in the vegetative phase. Healthy hyphae with normal clamps and mediate branches were always present in large number, and compared with these, the number of dead or damaged hyphae was insignificant. No narrowing of hyphae was noticed. (*b*) The main plate was examined for nearly two months but during this period no conidia had developed, nor was there any tendency of living hyphae to break up into oidia.	The reproductive phase, however, showed considerable damage. Fruiting areas appeared in the main plate on 25th July, 1936, *i.e.*, after an interval of thirty days while the control fructified after twenty one days. A moist spot developed at one spot near the periphery of the main plate and in the course of four to five days it assumed a yellow colour. But no porous area was formed. A smear examination from this condensed yellow area showed the presence of a good number of basidia which were all rudimentary and without any sterigmata or spores, mixed with quite a good number of chlamydospores of varied shape and dimension.	Fruit-formation, therefore, was not only delayed by nine days, but the development of any regular fruit was totally suppressed.

In the vegetative stage.	In the reproductive stage.	Observations.
(*c*) The number of chlamydospores decreased a little as the culture became old and many empty chlamydospores could be found.		

Results in the sub-cultures (*i.e.* the first vegetative generation)

Two subcultures were taken from the main plate—the first on 6th July, 1936, *i.e.,* five days after the removal of radium and immediately after damage was noticed in the main plate, and the second on 8th July, 1936, *i.e.,* two days after the first subculture. These two subcultures, therefore, represent the first vegetative generation.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. A little amount of damage in the form of hyphae with protoplasm broken up only at places was noticed for the first few days in both the sub-cultures. But later on, the damage totally disappeared and a smear-examination on 23rd July, 1936 showed complete recovery of the vegetative hyphae to the normal state. (*b*) No conidia developed. (*c*) Chlamydospores were present in moderately good number throughout.	Recovery was also noted in the reproductive phase. Moist spot developed on the wall of the plates in both the sub-cultures on 29th July, 1936, and on 31st July 1936 perfect porous areas were formed, so that fructification appeared in the first sub-culture after twenty-five days and in the second sub-culture after twenty-three days. The control fruited within twenty to twenty-five days. Fruiting areas were regular and sections showed basidia in close cluster bearing sterigmata and spores without any tramal hyphae elongations.	It should be noted that growth in the main plate stopped as soon as the hyphae reached the margin of the medium in the petri-dish. But in the sub-cultures growth was more vigorous. The hyphae not only ascended the walls of the plates but they could also overgrow the edges of the petri-dishes.

Recovery from damage

Damage was first noticed in the main plate on 6th July, 1936. Recovery was noted in both the plate subcultures (of the first vegetative generation) on 23rd July, 1936. The total period taken to recover was therefore 17 days.

Fruiting period of		Fruiting period in the control
(*a*) Main Plate (abortive fruit)	.. 30 days	21 days
(*b*) Sub-culture:–		
(1) 1st. Sub-culture (regular fruit)	.. 25 days	20-25 days
(2) 2nd. Sub-culture (regular fruit)	.. 23 days	

(3) A malt-agar plate-culture of *Polystictus leoninus* inoculated on 18th September, 1936, was subjected to 120 mg. radium for six days continually from 23rd September to 29th September, 1936. The radium was applied to the glass surface of the bottom part of the petri-dish containing the culture, which was kept inverted during the exposure. The hyphae were all with clamps and with a good number of short mediate branches arising at right angles from the main hyphae, were non-conidial with a few chlamydospores and did not show any trace of basidia or dead hairs when the exposure was started. A separate plate-culture was kept as a control.

Damage in the main plate

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae Damage to the vegetative hyphae became evident on the very first smear-examination on 26th September 1936, *i e.* after three days' exposure. The protoplasm of many vegetatative hyphae became much vacuolar and broke up at places and there was absolutely no trace	Basidia were first noted in the main plate on 29th September, 1936, so that rudimentary fruit-formation took place in the course of eleven days after inoculation. The control fruited perfectly well after six days. The fruiting area was not developed in a circular zone but formed in patches of toothed areas round the peri-	Not only the vegetative phase but the reproductive phase as well was extremely affected by such long exposure to heavy dose of radium. No erect fruit-bodies ever developed on the culture-plate.

In the vegetative stage.	In the reproductive stage.	Observations.
of basidia. After six days' exposure damage became more pronounced. Healthy hyphae almost disappeared and every hyphae showed the damaged character. The hyphae became much narrower than the normal ones, much more vacuolated, and broke up at places. As the culture grew old this broken up nature of the hyphae became more prominent and a strong tendency to break up into oidia was evident. A large number of healthy hyphae totally lost their clamps and cell-contents, and became converted into dead and double-walled hairs. The clamps became extremely reduced in size and many empty hyphae with such clamps, either wholly or partially empty but still attached, could be seen. But clamped hyphae always preponderated over such non-clamped hyphae. Extreme narrowness of the vegetative hyphae whether clamped or non-clamped was a noticeable feature. (*b*) A few conidia developed on 19th October, 1936, *i.e.* nearly a month after inoculation, but instead of increasing in number they altogether vanished. (*c*) Few chlamydospores that were found in the beginning disappeared entirely. No conidia or chlamydospores could, thus, be found in the main plate at the end.	phery of the culture, easily distinguished from the vegetative area by their yellow colour. The pores were very shallow and smear-examination from these areas showed a good number of basidia which never came to maturity. The basidia were very much smaller than the normal ones and shrunken in appearance, they were never in cluster, but always very irregularly scattered without showing anywhere any trace of sterigmata or spores. No tramal hyphae could be found amongst them. The basidia were present in good number at first but gradually they underwent disintegration.	

Damage in the plate sub-cultures (first Vegetative generation)

Altogether five subcultures were carried out from the main plate—the first on 26th September, 1936, after three days' radium exposure, the second on 29th September, 1936, after six days' radium exposure, the third on 5th October, 1936, six days after the removal of radium, the fourth on 26th October, 1936, twenty-seven days after the removal of radium and the fifth on 29th October, 1936, *i.e.*, thirty days after the removal of radium. The first four subcultures were in malt-agar plates and the last in malt-agar tube. These five subcultures may be broadly divided into two groups (I & II) so far as their characters are concerned, the first two comprising one group and the last three comprising another group.

In the vegetative stage.	In the reproductive stage.	Observations.
I. (*a*) Hyphae Damage caused by radiation upon the plate was fully transmitted to all the sub-cultures of the first generation. But the extent of damage varied in the two groups of sub-cultures. In the first and second sub-cultures comprising the first group, the vegetative hyphae were all damaged with protoplasm broken up into fragments, they were much shrunken and much narrower than the normal ones at places only. Nevertheless, a large number of hyphae could be found which though damaged, were as broad as the normal ones, but with protoplasm very much streaked in appearance. Hyphae were all damaged, but still clamped hyphae preponderated over the non-clamped hyphae. (*b*) A tendency to break up into oidia was not much apparent but a small number of	Fruit formation (as noted by the first appearance of basidia) occurred in the first and second sub-cultures after ten days, and in the third, fourth and fifth sub-cultures after twelve days. The fruiting period in the control varied at that time from six to seven days. As regards the reproductive phase, some differences could be noticed between the two groups of sub-cultures. In the first and second-sub-cultures a good number of basidia could be found arranged in rather sparse cluster, so that a tendency to form a hymenial layer was evident. No basidiospores were found. Erect fruits developed in both and were inserted in agar plates on 12th December, 1936. But no spore-fall occurred, and sections showed that their pores were filled with dead tramal hyphae and there was no trace of any basidia or living element.	It may be stated in general that damage to both the vegetative and reproductive phases was far greater (and almost to the same extent as in the main plate) in the case of the last three sub-cultures than in the case of the first and the second.

In the vegetative stage.	In the reproductive stage.	Observations.
conidia developed nearly a month after inoculation in both. the plates. Their number never increased but they rather finally vanished. (*c*) Chlamydospores developed in small number at first but increased to immense number later on. II. (*a*) Hyphae In the third, fourth and fifth sub-cultures, the conditions were rather different. Damage to the vegetative hyphae was rather more pronounced and no wider hyphae could be found as in the two previous sub-cultures. Hyphae were all extremely narrower than the normal ones and the sizes of the clamps were extremely small. The majority of the hyphae had no clamps, so that non-clamped hyphae were in preponderance over clamped hyphae. (*b*) A tendency to break up into oidia was more marked and, as a matter of fact, conidia appeared in all in the course of thirteen to fifteen days after inoculation, which is far more quicker than that in the previous case. (*c*) Chlamydospores developed in all, but contrary to the cases of the first and second sub-cultures the number of conidia went on increasing in immense number while the number of chlamydospores became reduced to a few only.	In the third, fourth and fifth sub-cultures toothed areas were formed but no erect fruit-bodies ever developed. Basidia could be found in smears only from the fruiting areas and even then, they were only a few in number, very irregularly scattered and very much shrunken in appearance. No sterigmata or spores were found.	

Results in the tube sub-cultures (second vegetative generation)

As the third, fourth and fifth subcultures of the first generation showed a decidedly greater amount of damage than the rest, they were again subcultured in malt-agar tubes on 2nd December, 1936, to find out how far the damage persisted.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. In this generation a partial recovery of the vegetative hyphae was noted. Hyphae were, on the whole, a little narrower than the normal ones and with clamps a little narrower in size, but the fragmented nature of the protoplasm was evident at some places only and not everywhere. Wider hyphae with protoplasm much vacuolated or streaked in appearance together with narrow hyphae filled up with protoplasm could be found in almost equal number with the damaged hyphae. Clamped hyphae came into preponderance and such clamped hyphae which were densely filled up with protoplasm, did not show any damage except for the fact that they were narrower than the normal ones. A good number of damaged hyphae became converted into hairs at the approach of the fructifying period. (*b*) A few conidia developed in all, though after seven days from the inoculation-date, but their number never increased. (*c*) Chlamydospores developed in small number.	Fruiting areas developed in all three sub-cultures of the second generation on 9th December, 1936, *i.e.*, seven days after inoculation, which falls within the normal period. The fruiting area was confined to the top of the slant in the form of a condensed yellow spot which afterwards formed pore-tubes. Smear-examination from these fruiting areas showed the presence of quite a good number of basidia arranged either in sparse cluster or irregularly scattered. But never were any sterigmata or spores found. However, three fruit-bodies from three tube sub-cultures were taken and inserted on the lid of an agar plate on 25th December, 1936. Absolutely no spore-fall occurred even after three days under moist bell-jar, and a section on 28th December, showed that the pore-tubes had a few rudimentary basidia here and there without any spore or living tramal hyphae.	It is thus evident that though there was a partial recovery of the vegetative phase in the second generation, there was no sign of recovery in the reproductive phase.

Results in the tube sub-cultures (third vegetative generation.)

As only partial recovery was noted in the second generation, the third, fourth and fifth subcultures were again subcultured in malt-agar tubes on 25th December, 1936.

In the vegetative stage.	In the reproductive stage.	Observations.
(*a*) Hyphae. Smear examination on 31st December, 1936 showed that the vegetative hyphae had completely reverted to the normal state. A few damaged hyphae could still be found in the third subculture but they resembled those that are found in old normal cultures. However, this damaged character did not persist long. Complete recovery of the vegetative hyphae was noted in all. The hyphæ became as broad as the normal ones and were filled up with protoplasm without showing any streaked, broken up or vacuolated character. A good number of vegetative hyphae became converted into hairs with the approach of the fruiting period. (*b*) Conidia altogether disappeared. (*c*) A small number of chlamydospores developed and they gradually increased in number. Increase of chlamydospores takes place also in normal cultures when they become old.	The first appearance of basidia was noted in smear examination on 4th January, 1937 in all, so that fruit-formation was noticed in all after ten days. The fruiting period in the control at that time was also ten days due to reduced room-temperature in winter. The basidia were arranged in sparse cluster and were never with any sterigmata or spores. Fruit-bodies showing regular pores developed on the tops of slants in all and three fruit-bodies of three subcultures were inserted on the lid of an agar plate on 9th January, 1937. No spore-fall occurred from any of them even after six days under a moist bell-jar, and a section on 15th January, showed that the pore-tubes had only a few undeveloped basidia mixed with a few tramal hyphae without clamps or spores. A peculiar feature was that a large number of chlamydospores was found within each poretube.	It is therefore seen that damage to the reproductive phase still persisted to a great extent in the third generation, although complete recovery in the vegetative phase was noted.

Recovery in the fourth vegetative generation

The third, fourth and fifth subcultures were again subcultured in malt-agar tubes on 7th January, 1937. The vegetative hyphae

did not show any damage. They were normal in all respects showing a good number of mediate branches. No conidia developed, but a few chlamydospores were found.

Fruit-formation was noted in all after eleven days on 18th January, 1937, the control also fruiting at the same time. The fruit-bodies formed were very regular, showing well-developed pore-tubes, and three fruit-bodies from three subcultures were inserted on the lid of an agar plate on 25th January, 1937. Spore-fall occurred within four hours of insertion and continued for three days upto 27th January, 1937. Sections showed pore-tubes densely lined with basidia bearing sterigmata and spores (agreeing with the normal ones in measurements) mixed with a few tramal hyphae bearing clamps and some tramal hyphae bearing terminal globular spores. Spores dropped from these three fruit-bodies of three subcultures were transferred to three malt-agar tubes on 27th January, 1937. Growth was vigorous in all of them and fruit-bodies appeared in them within ten days after the transfer.

Results in the tube sub-cultures (fifth vegetative generation)

Subcultures were carried for one generation more in malt-agar tubes. In no case was any damage noted either in the vegetative phase or in the reproductive phase and the cultures were normal in all respects. No conidia developed, though a few chlamydospores were found. Regular fruit-bodies appeared after ten days, which falls within the normal period.

Transfer to wood-blocks

Out of the five subcultures of the first vegetative generation from the main plate, the last three, *viz.*, the third, fourth and fifth subcultures were transferred to wood-blocks (mango-wood) on 30th November, 1936. The wood-block cultures, therefore, represented the second vegetative generation. The damaged nature of the hyphae persisted in all for more than three months, but ultimately all of them showed recovery and formed regular fruit-bodies. Recovery was noted in the third and fourth subcultures in wood-blocks on 15th March, 1937, *i.e.*, after 105 days, and in the fifth subculture in wood on 5th March, 1937, *i.e.*, after 95 days. It was, thus, a case of complete recovery. Conidia which developed in small number in all wood-blocks in the beginning, were later on entirely replaced by chlamydospores.

Small fruit-bodies appeared in the third subculture on 1st April, 1937, *i.e.*, 121 days after inoculation and 16 days after its recovery, in the fourth subculture on 27th March, 1937, *i.e.*, 117 days after inoculation and 12 days after its recovery; in the fifth subculture on 20th March, 1937, *i.e.*, 110 days after inoculation and 15 days after its recovery. The fruiting period for the control

in wood-block culture at the time was 14 days. The fruit-bodies were well formed showing regular pore-tubes, and three such fruit-bodies from three wood-block subcultures were inserted on the lid of agar plates on 16th April, 1937. Copious spore-fall occurred on 17th April, 1937, but it became less on 18th April, 1937, after which it gradually stopped. Sections on 19th April, 1937, showed pore-tubes lined with basidia some bearing sterigmata and spores mixed with a few tramal hyphae bearing clamps but no terminal spores. Spores were not transferred to any malt-agar tube, but the agar plates containing discharged spores were kept inside a moist belljar. Clamps developed after five days and smear-examination showed normal hyphae of *Polystictus leoninus* with mediate branches and chlamydospores.

Recovery from damage

Damage was noticed in the main plate on 26th September, 1936, after the plate had received a continuous 120 mg. radium exposure for three days. The damage persisted right through the first vegetative generation in malt-agar plates. Partial recovery of the vegetative hyphae occurred in the second vegetative generation in malt-agar tubes. Complete recovery of the vegetative hyphae was noted in third generation in malt-agar tubes on 31st December, 1936, *i.e.*, after 96 days, though the damage still persisted in the reproductive stage. The period for recovery in wood-block subcultures, as has been said, varied from 95 to 105 days.

Summary of results

	Fruiting period.		Fruiting period in the control.
No Regular fruit-bodies formed	(A) In Main Plate	11 days	6 days
	(B) In Sub-cultures :—		
	I. 1st. vegetative generation	10-12 days	6-7 days
	II. 2nd. „ „	7 „	7 days
	III. 3rd. „ „	10 „	10 days
Regular fruit-bodies formed	IV. 4th. „ „	11 „	11 days
	V. 5th. „ „	10 „	10 days

Recovery and Fruiting period in wood-block sub-cultures

(Fruiting period in the control wood-block culture—14 days)

Wood-block culture from	Inoculated on	Recovery on	Period of recovery from damage	Fruiting on	The whole fruiting period	Fruiting period calculated from the date of recovery
3rd. sub-culture	30-11-36	15-3-37	105 days	1-4-37	121 days	16 days
4th. sub-culture	30-11-36	15-3-37	105 days	27-3-37	117 days	12 days
5th. sub-culture	30-11-36	5-3-37	95 days	20-3-37	110 days	15 days

50 mg. Radium-exposure upon *Trametes cingulata* in culture for ten days

A full grown malt-agar plate-culture of *Trametes cingulata* inoculated on 9th April, 1936, was exposed to 50 mg. radium on 25th April, 1936, *i.e.*, after an interval of sixteen days from the inoculation date. The radium was this time placed direct upon the surface of the culture by removing the lid, and the radium capsules were removed from the culture on 5th May, 1936, after a continuous exposure of ten days. The hyphae were all with clamps and with a large number of short mediate branches and there were a large number of conidia and a small number of chlamydospores before the exposure was begun. A few empty hyphae were present and the distinction between zoned and non-zoned areas altogether disappeared.

Damage in the main plate

Smear-examinations were carried out daily both during the exposure and after the exposure had been stopped. With increasing exposures a large number of hyphae became damaged so that a large number of dead hyphae were produced. The protoplasm of

such hyphae became very much broken up into disconnected chains and a strong tendency to break up into oidia became more and more evident in the living hyphae. Clamps became extremely reduced in size wherever present, and the majority of the hyphae became extremely narrower than the normal ones and non-clamped. Conidia gradually increased in immense number and chlamydospores disintegrated in large number and almost disappeared. A large number of empty chlamydospores was found. The culture was not totally killed but dead hyphae ultimately preponderated over the living ones, all of which became distantly branched and showed the damaged nature distinctly.

Damage in plate sub-cultures (first vegetative generation)

Altogether five subcultures were carried out from the main plate during ten days of radium-exposure. The first subculture was after two days' exposure, the second after four days' exposure, the third after six days' exposure, the fourth after nine days' exposure and the fifth and the last after ten days' exposure.

Damage noticed in the main plate was transmitted to all the subcultures, but the extent of damage was far less. Damaged hyphae with broken up protoplasm or totally empty and dead hyphae were present in all in fairly large number but living hyphae preponderated in all. Moreover, the number of clamped hyphae was far greater than the non-clamped hyphae. Conidia were present in all together with a small number of chlamydospores.

The third, fourth and fifth subcultures were transferred to a dark room on 13th May, 1936, and kept in the dark thereafter. By keeping these three subcultures in the dark there was a sharp decline in the number of conidia, most of which developed a thick wall round them and became converted into chlamydospores. The number of chlamydospores so immensely increased that conidia almost vanished, while in the first and second subcultures kept in diffused light there was no reduction in the number of conidia and a few chlamydospores always remained. But such conversion into chlamydospores also takes place in the control kept in the dark, especially as the culture becomes old.

Transfer to wood-blocks (second vegetative generation)

Inocula from the third, fourth and fifth subcultures were transferred to sterilised wood-blocks (mango-wood) on 11th June, 1936. Examined on 8th August, 1936, they showed an equal proportion of damaged and healthy hyphae but the immense number of chlamydospores present in the plate subcultures of the first vegetative generation was entirely replaced by almost the same number of conidia.

No fruit-formation was noticed anywhere either in plates or in wood-blocks as it was a conidial non-fruiting strain.

Irradiation of freshly shed spores of *Polystictus leoninus* by 20 mg. radium for one hour

Fresh normal spores discharged from an artificial fruit-body of *Polystictus leoninus* were caught on two agar plates on 18th March, 1937. One plate was immediately exposed to 20 mg. radium, which was placed on the back of the plate so as to be nearer to the spores. The other plate containing spores was kept as a control. Irradiation was continued for an hour, after which the irradiated plate and the control-plate were both kept under a moist belljar in the culture room. On the next day, *i.e.*, 19th March, 1937, it was found that the irradiated as well as the control spores had all germinated. The irradiated spores were at once transferred to two malt-agar tubes and the spores from the control plate to another malt-agar tube. The tubes were kept in the diffused light of the culture room.

For two days no growth was noticed in any of the tubes. Growth was found to start in all on 22nd March, 1937, *i.e.*, on the third day after the transfer of spores. Linear growth was equal in all but the culture obtained from radiated spores was more fluffy than that from the normal spores. This condition persisted for the first few days after which growth in all three tubes became equal in nature. Fruit-formation appeared in the radium exposed tube-sub-cultures after twelve days and in the control-tube after thirteen days. The fruit-bodies were all regular showing well-developed pore-tubes, they were all (including the control-fruit) inserted in agar plates on 3rd April, 1937. The spore-fall was copious from all three fruits and occurred within three hours of insertion and continued for four days though showing a gradual decrease. The agar plates containing the discharged spores were kept under a moist belljar. Germination was quick and normal in all and clamps developed after five days in agar plates.

Mycelia from the three tubes just mentioned, of which the fruit-bodies had shed spores, were subcultured again on 31st March, 1937, in malt-agar tubes. The linear growth was the same and equally vigorous in all. Fruit-bodies appeared in all on 7th April, 1937, *i.e.*, after seven days and were very regular. Three fruits, one from each tube, were inserted again on 9th April, 1937, in agar plates for spore-fall; one of the fruit-bodies, as before, belonged to the control. Copious spore-fall occurred on the same day from all of them within three to four hours of insertion and continued for four days. No spore-fall occurred, however, during the day-time but only at nights. The agar plates containing the spores were kept under a moist belljar and all the spores germinated quickly. No further transfers of spores were carried out.

Smear-examinations, whether from the control-culture or from those obtained from germination of irradiated spores, did not show any difference, and all of them were quite normal in character.

RADIUM RAYS

Discussion and conclusions

It has been recorded by previous workers that strong doses of gamma-rays usually produce harmful effects on fungi and that especially heavy doses are necessary to produce lethal action. In the case of three Polypores treated with heavy doses of radium we had retardation of the vegetative growth, damage of the vegetative hyphae, suppression of spore-formation and delayed and imperfect development of fruit-bodies as described in the preceding pages; in some cases there was an increase in the number of chlamydospores. Ultimately, in subcultures (succeeding vegetative generations) recovery was noted within a varying period in all even where the radium was placed direct on the hyphae continuously for ten days, but it was usually found that recovery in the vegetative phase was quicker than in the reproductive one. Polypores, thus, seem to be extremely resistant to radium; Dauphin (6) exposing lower fungi to radium rays obtained sudden cessation of mycelial growth and of germination of spores but they were not killed because they began to grow again when brought to their normal condition. In the case of light doses of 5 mg. radium the damage was slight and recovery was very quick. Germination of spores was not affected in any way by exposing freshly shed spores of *Polystictus leoninus* to 20 mg. radium for one hour. Here no permanent change in the form of saltation or mutation could be produced; Lee, Haines and Coulson (11) could obtain besides lethal action only temporary inhibition of cell-division by exposing bacteria to 920 mg. of radium, they divided normally when they were removed from the radium rays and transplanted to fresh medium. Sibilia (15) has reported, however, two saltants from *Heterosporium gracile* by the action of radium rays, which have remained unchanged through several successive generations, though he was quite unsuccessful with ultra-violet radiation. It has been found that irradiation of the malt-agar medium with 15 mg. radium for half a minute had no toxic effect on growth of Polypores on it. Our experimental results show that *Polystictus leoninus* in majority of cases is more sensitive to radiation (sunlight, ultra-violet, X-rays and radium) than *Polyporus ostreiformis* or *Trametes cingulata.* These Polypores, I have found, usually fruit only in light, in complete darkness either they do not fruit at all or form in a few cases very imperfect and abnormal fruiting areas.

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