IV. *Studies in the Genera* Cytosporina, Phomopsis, *and* Diaporthe. VI.—*On the Conversion of one Strain of* Diaporthe perniciosa *into Another*.*

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[Plates 16–19.]

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I. Introduction.

An account of saltation in the older parts of cultures of *Diaporthe perniciosa* strain DH_C was given in an earlier paper (Horne and Das Gupta, 1929). The main features of this saltation were its occurrence in every cultural generation at a definite age; the gradual transformation of the whole parent mycelium into the saltant DH_F; the absence of visible indications of saltation in the body of the parent culture; and the inability of the saltant, which spreads relatively fast in fresh medium, to outgrow the slow-growing parent.

While attempting to discover if there were any change with age in the nature of the branching of DH_C hyphæ concomitant with the appearance of saltation, it was found that viable hyphal tips from the advancing mycelium of DH_C usually develop into DH_F. Very minute fragments of older mycelium were also found to give rise to DH_F when grown in fresh medium. Hence there was the possibility that DH_C might be an intimate association of hyphæ of two different characters DH_C and DH_F, with the DH_C dominating the form of growth of the latter. It was therefore thought advisable to make a careful study of the behaviour of DH_C and DH_F in both separate and associated culture. Preliminary experiments with individual cultures of DH_F showed that inocula taken from all parts usually bred true. On occasions, however, some inocula developed as mixed growth of the DH_C and DH_F, but no relation could be observed between the nature and position of inoculum and the occurrence of mixed growth. Attention was therefore devoted to the DH_C culture. The results presented in this paper relate chiefly to the behaviour of DH_C and the effect of inoculating DH_F with DH_C mycelium.

II. Material and Method.

The fungi used were the two strains DH_C and DH_F (Horne and Das Gupta, 1929) obtained in 1928 by saltation of *Diaporthe perniciosa,* and since maintained as stock cultures.

Medium.—A standard synthetic medium of the following composition was used: glucose 2·0 gm., asparagin 2·0 gm., $MgSO_4$ 0·75 gm., K_3PO_4 1·25 gm., starch 10 gm., agar 15 gm., distilled water 1,000 c.c. It should be noted that the phosphate is K_3PO_4 instead of the KH_2PO_4 used in earlier work (Horne and Das Gupta, 1929). The substitution was made because K_3PO_4 while in no way affecting the saltation promotes a more regular growth of the fungus than does KH_2PO_4. In experiments requiring a liquid medium the agar was omitted.

Method. (*a*) *General inoculation procedure.*—A number of standard-medium plates were inoculated from a stock culture. After five or six days the cultures judged most suitable were chosen for inoculating the plates of the experimental series described later. Inocula which were approximately 1 cu. mm. in size were taken from the

compact edge of DH_F cultures and from the region 2–3 mm. within the diffuse margin of DH_C cultures.

(*b*) *Special inoculation procedure.*—Special methods devised for particular experiments are described in appropriate places.

All cultures were kept in an incubator at 20° C. unless a different temperature is definitely mentioned.

III. Growth Characteristics of DH_C and DH_F.

A comparison of the macroscopic and microscopic characters and of the rates of spread of DH_C and DH_F in the standard medium is given in some detail since this paper deals with changes in these characters induced by various factors.

(*a*) *Macroscopic Character.*

DH_C.—The mycelium is white; a considerable proportion grows submerged and some may adhere to the bottom of the plate. Prostrate hyphæ form a thick, compact growth with a conspicuous *absence of aerial mycelium and of zonation.* Very fine, narrow zones appear in older cultures, but are visible only by transmitted light. The *margin is irregular and indefinite in young* cultures, but *regular and well defined in the old.* The fungus is of a *strongly-staling* type. A photograph of an eleven days' old culture is given in fig. 14, Plate 16, and of one thirty-one days' old in fig. 15, Plate 16.

DH_F.—The mycelium is white and mostly *superficial.* Wide zones of prostrate hyphæ which appear as radiating strands, particularly by transmitted light, alternate with narrow rings of erect, aerial mycelium. The *margin is always regular and well defined.* The fungus is of a *non-staling* type. A photograph of an eleven days' old culture is given in fig. 13, Plate 16.

(*b*) *Microscopic Character.*

DH_C.—The marginal hyphæ in young cultures are sparse and haphazardly arranged; those in very old cultures are quite uniform with their tips all lying on the same circumference. In young cultures some hyphæ grow far in advance of the rest and are thin with one or two branches only, fig. 16, Plate 17; a few become thick and globular at the tip and disintegrate. *Each individual hypha produces few lateral branches.* Branches are long and thin and form an acute angle with the main hypha, fig. 1, A.

DH_F.—The *marginal hyphæ* in both *young and old* cultures are *compact* with their tips lying almost all on the same circumference, fig. 17, Plate 17. *Each individual hypha is profusely branched.* Branches are short and stout; they form an acute angle with the main hypha and three or more may arise successively on the same side of the hypha, fig. 1, B.

Fig. 1, A and B. Diagram illustrating the branching of the two strains. A. DH_C. B. DH_F.

(c) *Rate of Spread.*

DH_C.—Relatively slow, fig. 2. *DH_F*.—Relatively fast, fig. 2.

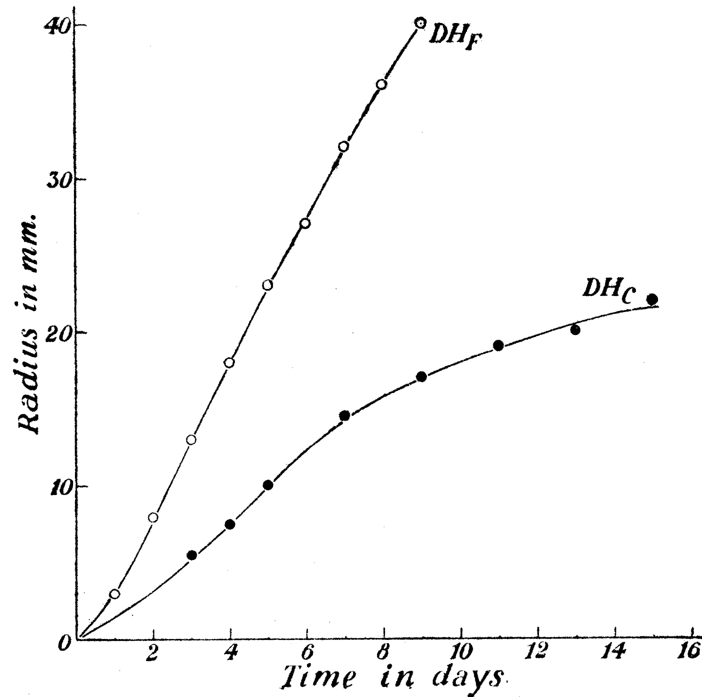

Fig. 2. Graphs showing the rate of spread of DH_C and DH_F in standard medium at 20° C.

IV. Development of DH_C and DH_F from Fragmented Mycelium of DH_C.

The difference in types of growth arising respectively from large and small inocula and from fragments of individual hyphæ is dealt with here for strain DH_C.

1. *Relation between Size of Inoculum and the Nature of Subsequent Growth.*

Types of growth arising from inocula approximately 1 *cu. mm. in size.*—Successive inocula, approximately 1 cu. mm. each, were taken radially from the centre to the margin of DH_C cultures of various ages. The method is fully described in a previous paper (HORNE and DAS GUPTA, 1929). It was found that inocula containing young DH_C mycelium always produce DH_C, but inocula of old DH_C mycelium from the central region of any DH_C culture exceeding 15 mm. in radius, produce DH_F. The result here is slightly at variance with that given in the earlier paper, where the phenomenon is described as saltation; DH_C appears to have become rather more stable and to saltate when the mycelium is comparatively older.

Types of growth arising from minutely fragmented mycelium of DH_C.—The experimental method adopted was as follows: A small inoculum of DH_C was transferred to a drop of sterile water on a sterilized slide and cut with a lance-shaped needle into very fine fragments which were then washed into a test-tube containing 25 c.c. of sterile water and shaken into a suspension. Equal quantities, about 2 c.c., of the suspension were added to separate tubes containing standard medium previously melted and cooled to about 40° C. The contents of each tube were then poured into separate plates. This operation is hereafter referred to as "plating." The number and type of colonies produced were recorded usually after five days' growth.

Inocula of young mycelium from the margin of DH_C cultures of different age were treated as described above. The number of DH_C and DH_F colonies arising from the fragments, together with other details of the experiments, are given in Table I.

TABLE I.—Number of DH_C and DH_F Colonies Produced from Minute Fragments of Young Mycelium taken from DH_C Cultures of Various Age.

Exp.	Culture employed.		Position in culture of inoculum taken.	Approx. size of inoculum, cu. mm.	No. of plates used.	Number of colonies.		
	Age, days.	Radius, mm.				DH_C.	DH_F.	DH_C+DH_F.
A	10	18·0	Margin	1·0	10	1	7	—
B	10	18·0	,,	1·0	10	4	18	—
C	7	14·0	,,	1·0	10	5	28	—
D	5	10·0	,,	1·0	6	2	27	—
E	3	6·0	,,	1·0	6	5	20	—
F	1	1·5	,,	1·5	6	5	7	—
G	1	1·0	,,	1·0	6	6	2	13
H	1	1·0	,,	1·0	6	4	4	7
J	1	0·5	,,	0·5	6	28	—	10
K	1	0·5	,,	0·5	6	29	—	4

The point of outstanding interest in Table I is that in all but two experiments, fragments of young DH_C mycelium produced a number of pure DH_F colonies besides a few of their own type. In experiments J and K no pure DH_F, but several colonies of DH_C mixed with DH_F arose. In the first five experiments the number of DH_F far exceeded that of DH_C colonies. Inocula from very young cultures (Table I, F–K) showed a decrease in the number of DH_F colonies. The absence of pure DH_F and the appearance of mixed colonies in experiments J and K may have been due to inadequate separation of the two types of hyphæ in the process of cutting.

Inocula of old mycelium from the central region of DH_C cultures of three different ages were crushed and plated. The types of growth and the number of colonies arising from the fragments, together with details of the experiments, are given in Table II.

TABLE II.—Number of DH_C and DH_F Colonies Produced from Minute Fragments of Old Mycelium taken from DH_C Cultures of Various Age.

Exp.	Radius of culture employed, mm.	Position in culture of inoculum taken.	Approx. size of inoculum, cu. mm.	No. of Plates used.	Number of colonies.	
					DH_C.	DH_F.
A	15·0	Centre	0·2	10	—	69
B	13·0	,,	0·2	10	—	47
C	9·0	,,	0·2	10	5	27

It will be seen from Table II that again a number of DH_F colonies developed from fragments of DH_C mycelium. A special point of interest here is the complete absence of DH_C colonies in the first two experiments and the appearance of only five in the third. Altogether there were five DH_C colonies to 143 of DH_F.

A few similar experiments were made with different parts of DH_C cultures. Inocula (approximately 1 cu. mm. each, and referred to as A_1, A_2, . . . A_{16}) taken successively from periphery to centre of a DH_C culture of 16 mm. radius, were crushed and plated separately, each being distributed over ten plates. The number of colonies developing from A_{16}, A_{15}, . . . A_{10} was too great to permit of a reliable count. The colonies from A_1, A_2, . . . A_9 were less numerous and the count of the total number of colonies in the ninety plates containing fragments of these inocula showed the presence of 714 colonies of DH_F to 59 of DH_C.

Since the number of colonies developing from fragmented inocula of an old DH_C culture was so high, it was decided to test a small culture of 3 mm. radius. Successive fragments (1 cu. mm. each) from the periphery to the centre, here referred to as B_1, B_2, and B_3, were crushed and plated separately. Details of the experiment and the number of colonies of each type are given in Table III.

TABLE III.—Number of DH_C and DH_F Colonies Produced from Fragments (B_1, B_2, B_3) from Periphery to Centre of a DH_C Culture of 3 mm. Radius.

Position in culture of inoculum.	Approx. size of inoculum, cu. mm.	No. of plates used.	Number of colonies.	
			DH_C.	DH_F.
B_1-periphery	1·0	6	7	11
B_2-intermediate	1·0	6	4	16
B_3-centre	1·0	6	3	30

Table III shows that more colonies developed from the older than from the younger part of the culture, and further that the resulting DH_F colonies were more numerous than those of DH_C.

The possibility that the temperature of the melted medium at the time of adding the suspension may be responsible for the appearance and preponderance of DH_F cultures from plated fragments of DH_C hyphæ was investigated by a modification of the previous method.

A suspension of DH_C fragments was made in the usual manner, but instead of being added to the melted medium, equal portions of it were poured on to the surface of solid standard medium contained in ten separate plates. The suspension was spread by tilting the plates.

Inocula from the margin of DH_C cultures which were respectively seven and five days old were treated in this way. The numbers of DH_C and DH_F colonies produced are given in Table IV.

TABLE IV.—Number of DH_C and DH_F Colonies Produced from Minute Fragments of Young Mycelium taken from DH_C Cultures of Various Age. (*Possible Temperature Effect Eliminated.*)

Exp.	Age of culture employed, days.	Position in culture of inoculum.	Approx. size of inoculum, cu. mm.	No. of plates used.	Number of colonies.	
					DH_C.	DH_F.
A	7	Margin	1·0	10	2	37
B	5	„	1·0	10	9	30
C	5	„	1·0	10	7	20

It may be seen from Table IV that once more DH_F developed from fragmented DH_C mycelium and that the proportion of DH_F to DH_C colonies was 87 : 18, indicating that neither the appearance of DH_F nor the paucity of DH_C colonies recorded in the

previous experiments was due to the temperature of the medium when the suspension was added.

Since it was also thought possible that the actual crushing of the hyphæ might account for the results, this operation was eliminated from later experiments. The method adopted was practically the same as that used in previous work on saltation (HORNE and DAS GUPTA, 1929). Successive portions of the smallest possible dimensions were removed from a DH_C culture starting at the extreme edge and working radially towards the centre. The operation was performed with a fine lance-shaped needle under a dissecting microscope. The fragments were transferred in serial order to fresh medium and the type of growth developing from each indicated the nature of the mycelium at the corresponding place in the parent culture at the time when the fragments were removed.

Tests were restricted to the advancing region of comparatively young cultures. The number of fragments per mm. of radial distance varied, the maximum being in the experiment in which twenty-six fragments were obtained from a strip 2 mm. long. The fragments were numbered consecutively from the margin inwards, and are referred to by their numbers. Table V embodies the results of three experiments.

It may be observed in Table V that minute fragments from a short strip extending radially inwards from the margin of one DH_C culture grew into DH_C or DH_F or a mixture of the two ; fragments of another culture gave rise almost exclusively to DH_F ; and those from a third produced no DH_C at all. These results agree with those obtained by plating, and the further multiplication of examples is regarded as unnecessary.

2. *Relation between Types of Hypha and the Nature of Subsequent Growth.*

The appearance of two types of growth (DH_C and DH_F) from fragmented DH_C mycelium indicated the existence of corresponding characters in the parent culture, possibly in different hyphæ. The lack of uniformity in the hyphæ of the advancing edge of DH_C also lent support to this view. Accordingly, experiments were made in which fragments taken from individual hyphæ were grown separately and their respective growth-character recorded. The method used for cutting the hyphæ was as follows: Medium was spread thinly on a glass slide, which was then inoculated in the centre with a small fragment of DH_C mycelium and placed in a moist chamber. When the hyphæ began to grow, a number of them were sketched under the low power of a microscope. They were then cut into small fragments by means of a lance-shaped, iridio-platinum needle with a sharp cutting edge and each fragment was transferred to a petri-dish. The work was facilitated by the diffuse nature of the early growth. The hyphæ employed were either unbranched or had one or many, long, thin branches. Each main hypha was usually divided into a number of fragments by successive cuts so that some fragments included no branch, while others included one, two, or many branches, fig. 3. The results of the experiments are given in detail in Table VI.

TABLE V.—Types of Growth Arising from Minute Fragments Removed Radially from the Advancing Zone of DH_C Cultures of Various Age.

($C = DH_C$; $F = DH_F$; M = Mixture of DH_C and DH_F ; — = No growth.)

DH_C culture employed.		Dimensions of strip tested, mm.	Fragments from strip.		Position of fragments numbered consecutively from periphery to centre.																									
Age, days.	Radius, mm.		Number.	Approx. size, mm.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26
4	4·0	2·0 × 0·1	26	0·08 × 0·1	—	F	—	C	M	C	F	F	C	F	F	M	F	F	—	C	—	—	—	C	C	F	F	M	C	C
2	2·0	2·0 × 0·1	16	0·12 × 0·1	—	—	F	F	—	F	F	—	—	C	F	F	F	F	F	M										
1	0·5	0·5 × 0·1	8	0·06 × 0·1	—	F	—	F	F	F	F	—																		

TABLE VI.—Number and Nature of Cultures Arising from Severed Fragments (Grown Separately) of Individual, Young DH_C Hyphæ.

Fragments used.		No. of fragments producing			% of fragments.	
Branching.	Number.	DH_C.	DH_F.	$DH_C + DH_F$. (mixed)	Developed.	Disintegrated.
Unbranched	190	7	27	1	18·4	81·6
With one branch	99	3	13	0	16·2	83·8
With two branches ...	61	3	12	2	28·0	72·0
With many branches ...	106	11	28	0	37·0	63·0

Several interesting points may be noted in Table VI. The type of growth produced is independent of the branching of the fragment; the number of fragments producing DH_F was considerably greater than the number producing DH_C; a mixed growth of DH_C and DH_F occurred on three occasions, including once from an unbranched hyphal tip which possibly indicates the presence of a mixture of DH_C and DH_F characters at one point in the same hypha; a considerable number of fragments failed to grow. There is some indication that the mortality is greatest among fragments with one or no branch and least among the much-branched ones.

A selection of the drawings showing the position in the hyphæ of the fragments developing into DH_C and DH_F is given in fig. 3.

Fig. 3 reveals the remarkable facts that DH_C and DH_F may develop from different minor branches of the same hypha or even from contiguous fragments of one hypha. It may also be seen that those fragments which developed into DH_C usually included the extreme tip of a hypha, but there was no very marked consistency in the position of fragments giving one strain or the other, since DH_F also grew from the tip of a hypha and DH_C on a few occasions from the region behind the tip.

A slightly modified method was adopted in a further series of experiments. The culture of DH_C was prepared on a slide as before and the hyphal tip was cut off. Instead of removing the severed tip, however, this was left to grow on the slide, while the whole of the rest of the mycelium was transferred to a plate of standard medium as a control. It was thus possible accurately to sketch the severed tip under a high magnification by means of a camera lucida, and to measure it from time to time, while avoiding any injury associated with its transference to a plate. Particular attention was paid to the length of the hyphal tip severed and to its morphological characteristics.

Altogether forty-five tips were tested. These could be broadly divided into two types, viz., thick hyphæ with a number of short, thick branches, and thin hyphæ, unbranched or with one or more similarly thin branches. Out of the total of forty-five tips, twenty-six developed into DH_F, two into DH_C, and one into a mixture of the two strains. The remaining sixteen failed to grow and finally disintegrated, although

tests were confined to hyphæ in an apparently vigorous condition. Tips producing DH_C were of the thin type, as was also the one from which the mixture originated;

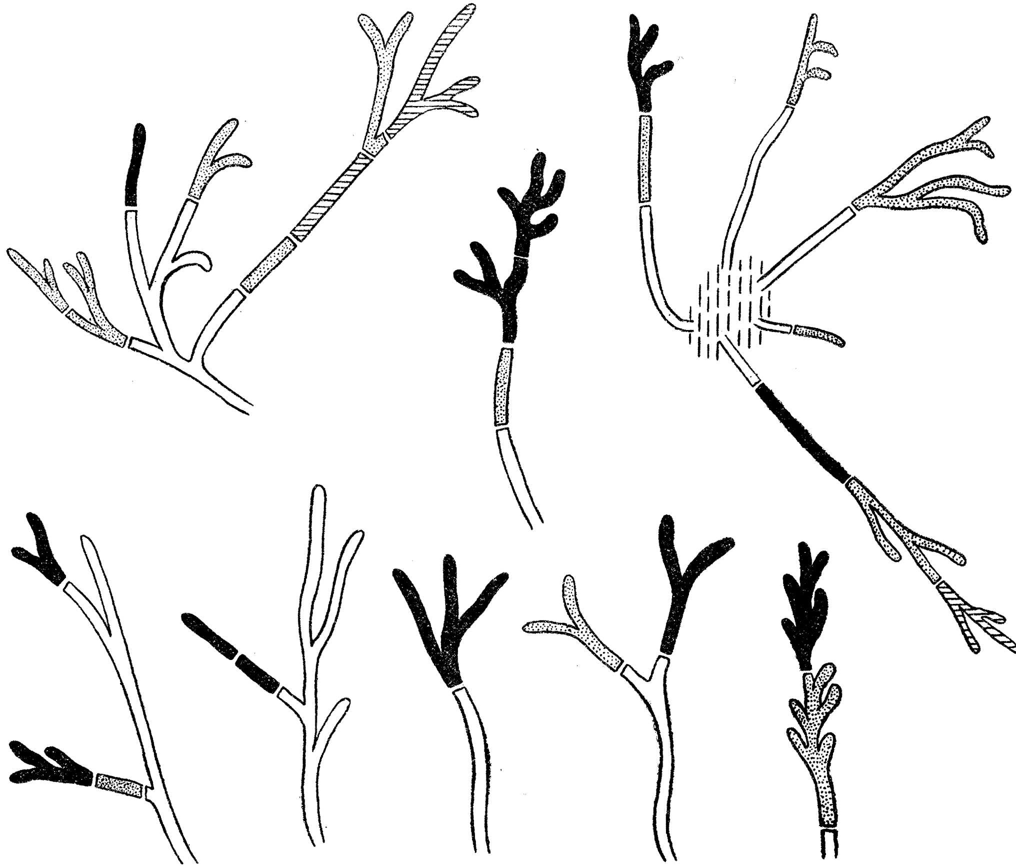

FIG. 3.—Semi-diagrammatic representation of hyphæ from a DH_C culture showing the positions of fragments and the nature of the growths obtained from them. The black, dotted and striped portions represent DH_C, DH_F and disintegrating fragments, respectively.

those giving DH_F were either thick or thin, fig. 4. Owing to the fact that so few DH_C cultures were obtained, no relation could be established between the type of hypha and the nature of its subsequent growth.

Representatives of both thick and thin hyphæ disintegrated, the first sign of the process being a shrinkage in length observable twelve to fourteen hours after cutting. The breakdown of a tip cut very small may possibly have been due to the absence of a nucleus, but this can hardly apply to disintegration of those tips which included a number of branches, since staining of DH_C hyphæ has revealed the presence of a delimiting cross-wall at the base of each branch.

The smallest tip found to grow was 0·2 mm. in length. No unusual features were noticeable in the manner of growth; each tip elongated and produced new branches which branched in their turn. One instance occurred of a secondary branch which outgrew the hypha that produced it and continued as the main branch. So far no

tip has been observed to elongate at the severed end, but in one experiment, when the tip of a hypha was removed, the severed end of the remaining part forked into two branches.

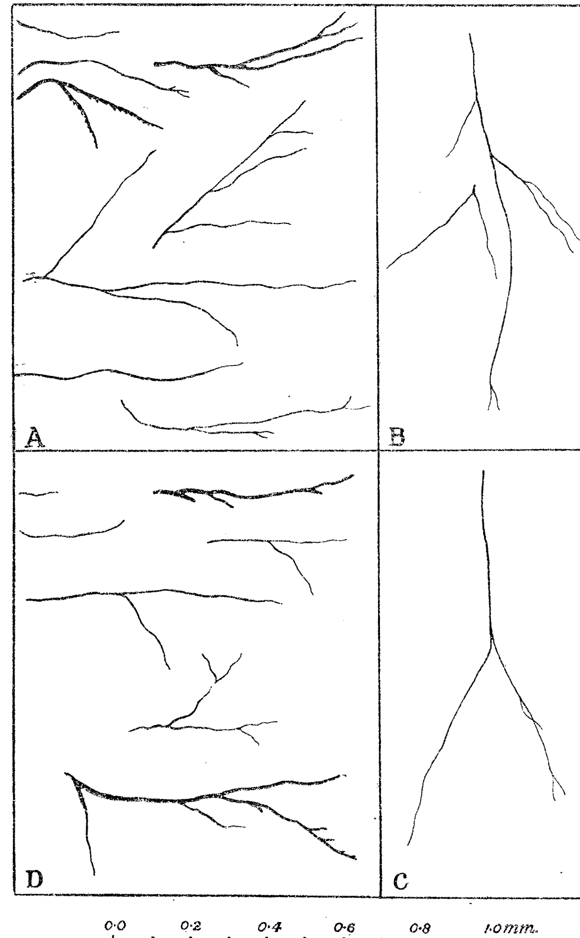

FIG. 4, A-D.—A. Hyphal tips which developed as DH_F. B. Hyphal tips which developed as DH_C. C. Hyphal tip which developed as a mixture of DH_C and DH_F. D. Hyphal tips which disintegrated.

V. CONVERSION OF DH_F BY DH_C.

1. *DH_F Inocula in Association with DH_C Inocula.*

With a view to ascertaining the influence of DH_C on DH_F suggested by the inability of DH_F hyphæ to exhibit their presence in a DH_C culture, experiments were conducted in which both strains, either mixed together or arranged at a definite distance apart, were placed on the same plate.

DH_C and DH_F Inocula mixed.—A small cube of a DH_C culture from the region 2 mm. inwards from the margin and another cube from the edge of a DH_F culture were placed on a sterile glass slide. The cubes were cut into small pieces with a lance-shaped needle, and transferred as one inoculum to the centre of a standard medium plate. Inocula were prepared to contain either approximately 1 cu. mm. DH_C culture mixed with

approximately 1 cu. mm. DH_F culture, or of 0·25 cu. mm. DH_C mixed with 0·5, 1·0, or 1·5 cu. mm. DH_F. Twelve inocula of each type were prepared and each was placed at the centre of a separate plate. Similarly fragmented cubes of each fungus were grown alone as controls. The cultures were measured at intervals and the average radius of spread of the sets of twelve replicates and of the DH_C and DH_F controls are given in Table VII.

TABLE VII.—Nature and Radius (mm.) of Cultures Derived from Mixed Inocula of Young DH_C and Young DH_F.

Approx. vol. of components of inoculum, cu. mm.		Ratio.		Interval of days.				Type of growth.
DH_C.	DH_F.	DH_C.	DH_F.	3.	4.	5.	7.	
0·0	1·0	0	1	9·0	14·0	20·0	30·0	DH_F
1·0	0·0	1	0	3·0	6·0	9·0	16·0	DH_C
1·0	1·0	1	1	4·8	6·5	10·0	16·5	DH_C
0·25	0·5	1	2	4·5	6·0	9·5	16·5	DH_C
0·25	1·0	1	4	3·0	5·0	9·0	15·0	DH_C
0·25	1·5	1	6	3·0	5·5	10·5	17·0	DH_C

It should be noted that both the growth rate and the morphological character of cultures from the mixed inocula are indistinguishable from those of DH_C alone. Further, mycelium from various parts of these cultures tested on the seventh day proved to be pure DH_C.

Similar experiments were made by mixing an inoculum containing young DH_C mycelium with one from the oldest part of a twenty-one days' growth of DH_C containing mycelium which is known to grow as DH_F. The inocula were mixed in various proportions. The radii of the resulting cultures were measured periodically and are given in Table VIII together with other details of the experiment.

Table VIII indicates that in both rate of spread and morphological character growths from inocula of young DH_C mixed with old DH_C (virtual DH_F) are indistinguishable from those of young DH_C alone.

DH_C and DH_F inocula in contact.—For this experiment, each of a number of standard medium plates was inoculated at four points which were about 3 cm. from the centre and at approximately equal distances apart. Two diametrically opposite inocula were each composed of a cube from the region 2 mm. inwards from the margin of a DH_C culture, in contact with a cube from the edge of a DH_F culture; the other two inocula, one of DH_C alone, the other of DH_F alone, acted as controls. Components of the attached inocula were either of equal size (approximately 1 cu. mm. each) or of unequal

TABLE VIII.—Nature and Radius (mm.) of Cultures Derived from Mixed Inocula of Young DH_C and Old DH_C.

Approx. vol. of components of inoculum, cu. mm.		Ratio.		Interval of days.				Type of growth.
DH_C, young.	DH_C, old.	DH_C, young	DH_C, old.	3.	4.	5.	7.	
0·0	1·0	0	1	0·0	6·0	15·0	21·5	DH_F
1·0	0·0	1	0	3·0	7·5	11·0	13·0	DH_C
0·25	0·5	1	2	5·0	9·5	11·5	13·5	DH_C
0·25	1·0	1	4	5·6	9·5	11·6	13·6	DH_C
0·25	1·5	1	6	5·7	9·5	11·5	13·7	DH_C

size (DH_C approximately 0·25 cu. mm. and DH_F 0·5, 1·0, or 1·5 cu. mm.). The resulting growths were measured periodically. The average radius of growths from the combined and from the control inocula and other experimental details are given in Table IX.

TABLE IX.—Radius (mm.) of Cultures Derived from Inocula of Young DH_C in Contact with Young DH_F, and of the Controls.

Approx. vol. of components of inoculum, cu. mm.		Ratio.		Interval of days.			
DH_C.	DH_F.	DH_C.	DH_F.	2.	5.	7.	9.
0·0	1·0	0	1	5·0	13·0	19·0	25·0
1·0	0·0	1	0	3·5	8·0	11·0	13·0
1·0	1·1	1	1	3·0	7·5	11·0	14·0
0·25	0·5	1	2	3·5	8·5	11·5	13·5
0·25	1·0	1	4	3·7	8·7	12·5	14·5
0·25	1·5	1	6	4·2	8·8	12·5	14·5

Table IX shows that the rate of spread of cultures arising from DH_C and DH_F inocula in contact with each other, whatever the relative size of the two, was the same as that of the DH_C control. From their external character and from the results of tests of mycelium from various parts, these cultures were pure DH_C. Hence, here again, DH_F is shown to be converted into DH_C by the influence of DH_C.

In further experiments an inoculum containing young DH_C mycelium was placed in contact with one of equal or larger size, containing mycelium from the oldest part of a DH_C culture which was twenty-one days old, *i.e.*, was virtually DH_F. The radius of the subsequent growth (average of 16 cultures) is given in Table X.

TABLE X.—Radius (mm.) of Cultures Derived from Inocula of Young DH_C in Contact with Old DH_C, and of the Control.

Approx. vol. of components of inoculum, cu. mm.		Ratio.		Interval of days.			
DH_C, young.	DH_C, old.	DH_C, young.	DH_C, old	2.	5.	7.	9.
0·0	1·0	0	1	0·0	6·0	15·0	21·0
1·0	0·0	1	0	3·0	7·5	11·0	13·0
1·1	1·1	1	1	3·0	7·0	10·5	13·0
0·25	0·5	1	2	3·3	7·0	10·0	13·0
0·25	0·75	1	3	2·3	6·7	9·5	14·0
0·25	1·0	1	4	3·0	7·0	10·5	13·5

It may be seen from Table X that the rate of spread of growths from the combined inocula was identical with that of the DH_C control. The external characters and mycelial tests further proved these cultures to be pure DH_C.

Experiments were also made in which an inoculum containing young DH_C mycelium was placed on the top of another containing young DH_F, and *vice versa.* On these occasions, too, mycelial tests, rate of spread, and the appearance of the cultures which developed indicated that they were pure DH_C.

It is evident, therefore, that the growth from a DH_C inoculum converts that from a DH_F inoculum into DH_C.

DH_C and DH_F inocula at various distances apart.—Each member of a series of plates, was inoculated with a pair of inocula, one DH_C and the other DH_F, which were placed at a definite distance from each other. The distances between the inocula were 2, 4, 6, 8, 10, 15, 20, 30, 40, 50, and 60 mm. Six replicates of each were made ; some were kept at 12° C. and the rest at 20° C. The growths which developed were measured periodically and any peculiarities in morphological character were noted.

When the inocula of DH_C and DH_F were 2, 4, 6, or 8 mm. apart, the two growths mingled to form a culture resembling one of DH_C, and tests of mycelium from different parts showed that the whole culture was this strain alone. Growths from pairs of inocula 10, 15, or 20 mm. apart mingled in a way which left no trace of the junction and tests showed that the DH_F mycelium, with the exception of that in the part most removed from the DH_C inoculum, had been converted into DH_C. Growths from inocula 30 or 40 mm. apart mingled to leave a faint line of demarcation and a similar but more pronounced line was visible when the distance between the inocula was 50 or 60 mm. Mycelial tests of these cultures proved that the DH_F on the side nearer to the DH_C inoculum had changed into DH_C.

The diameters of some of the cultures after four days' growth at 20° C., and of others after three and five days at 12° C. are shown graphically in fig. 5.

It appears from fig. 5 that the growth rate of DH_C was unaffected by its proximity to DH_F since all three graphs for DH_C are almost horizontal lines and such deviations as occur are slight, and probably due to the fact that an overgrowth of DH_F frequently prevented accurate measurement by obscuring the margin of DH_C.

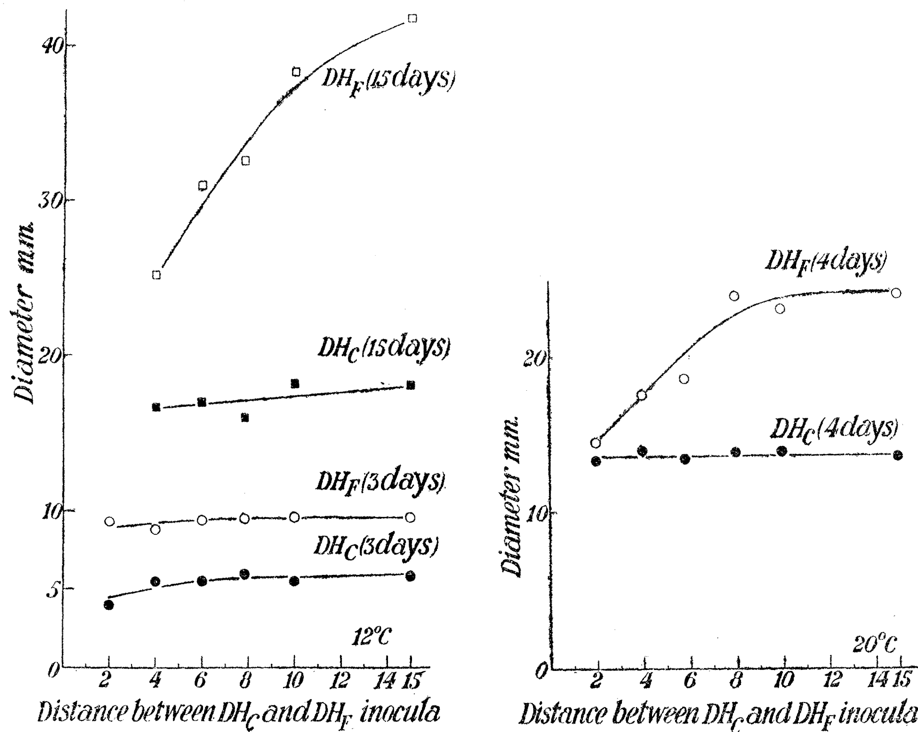

FIG. 5.—Graphs showing growth of DH_C and DH_F in relation to time and to distance between the two inocula.

The graph for DH_F after three days at 12° C. is also a horizontal line, but those after fifteen days at 12° C. or after four days at 20° C. slope steeply upwards from left to right indicating that, after a certain time, development of a DH_F inoculum placed near to DH_C was retarded to an extent which varied inversely with the distance between the inocula of the two strains. It should be particularly noted that this retardation occurred only after contact between the DH_F and DH_C mycelia had been established. Discontinuities in the curves of growth after the longer intervals of time may also be ascribed to the influence of DH_C after intermingling of the two strains.

2. *Established DH_F Cultures Inoculated with DH_C.*

Since all the mixed inocula of DH_C and DH_F developed into cultures of DH_C only, experiments were performed to determine the effect of inoculating established cultures of DH_F with DH_C.

DH_F inoculated with DH_C at the periphery.—Cultures of DH_F of different sizes were each inoculated at the *periphery* with *young DH_C mycelium.* The radii of the DH_F cultures before and after inoculation with DH_C were measured periodically along a

diameter at right angles to the one passing through the marginal inoculation. The values of the measurements and the types of growth obtained are given in Table XI, and certain results are shown graphically in fig. 6.

TABLE XI.—Radius (mm.) and Nature of DH_F Cultures After Inoculation with DH_C at the Periphery.

Radius when inoculated with DH_C, mm.	Number of days after inoculation with DH_C.											Character 12 days after inoculation with DH_C.
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	12.	
3·5	5·0	7·5	10·0	12·0	15·0	17·0	19·0	21·0	23·0	25·0	27·0	DH_C
4·5	6·0	8·0	10·5	13·5	16·0	18·0	20·0	22·0	23·5	25·0	27·0	,,
5·5	7·0	10·0	12·5	15·5	17·5	19·5	21·5	23·5	25·5	27·5	30·5	,,
6·5	9·0	13·0	15·5	18·5	21·0	23·0	25·0	26·5	28·0	29·5	31·5	,,
7·5	12·0	15·5	17·0	19·0	21·0	23·0	25·0	27·0	29·0	31·0	33·0	,,
7·5	12·0	14·5	17·0	20·5	23·0	25·5	27·0	30·5	33·0	35·0	37·0	$DH_C + DH_F$
9·5	14·0	19·0	23·0	26·0	29·0	32·0	35·0	38·0	41·0			,,
11·0	16·0	21·5	23·5	29·5	34·0	37·0	40·5	44·0	47·5			,,
14·0	17·0	22·0	25·5	29·0	32·0	34·5	38·0	41·5				,,
18·5	22·5	27·0	30·0	33·5	37·0	40·0	43·5					,,

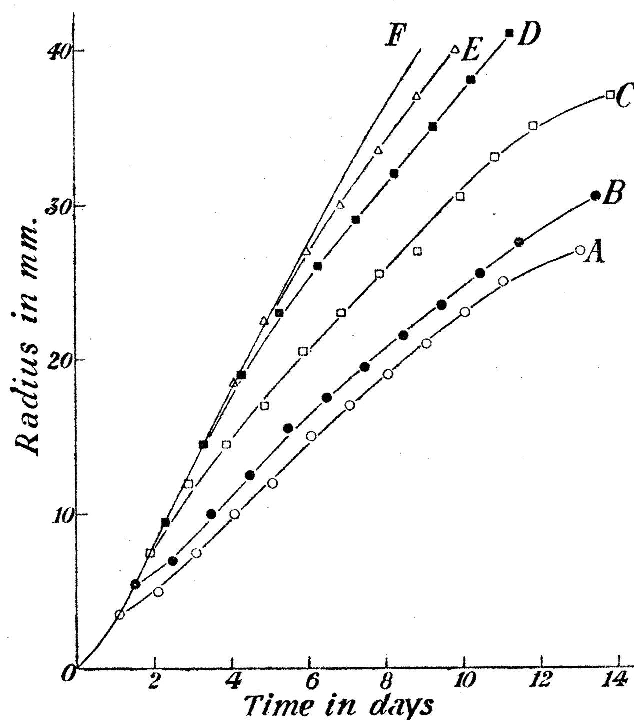

FIG. 6.—Graphs showing change in rate of spread of DH_F cultures after inoculation with DH_C mycelium at the periphery. A. Culture inoculated with DH_C when of 3·5 mm. radius. B. Culture inoculated with DH_C when of 5·5 mm. radius. C. Culture inoculated with DH_C when of 7·5 mm. radius. D. Culture inoculated with DH_C when of 9·5 mm. radius. E. Culture inoculated with DH_C when of 18·5 mm. radius. F. Culture not inoculated with DH_C.

Fig. 6 shows that after addition of DH_C the rate of spread of DH_F cultures not exceeding 7·5 mm. in radius at the time of peripheral inoculation with DH_C was immediately reduced from 4-5 mm. per day, normal for DH_F, to the value (about 2 mm. per day) typical of DH_C cultures. The growth of larger cultures (8-14 mm. radius) was also retarded, but to a less extent since the rate of spread fell less suddenly and then only to about 3 mm. per day, fig. 6, curve D. Very large cultures (18 mm. radius) continued to spread at the DH_F rate after peripheral inoculation with DH_C.

The relative positions of the different curves in fig. 6 were determined not only by the degree to which growth was retarded after addition of DH_C, but also by the length of the initial period during which DH_F was growing alone.

Mycelium taken twenty-four hours after marginal inoculation of DH_F with DH_C, from any part of a DH_F culture which had not exceeded 7·5 mm. in radius at the time of this inoculation, developed into DH_C. Similar tests of larger DH_F cultures showed that while the region nearer to the DH_C inoculum had been completely converted into this strain the parts further removed were still DH_F. Mycelial tests of a DH_F culture inoculated with DH_C when the radius was 25 mm. and tested three days later proved that only mycelium in the region extending 2 mm. round the periphery on each side of the DH_C inoculum had been converted. When another culture, inoculated with DH_C when of radius 32 mm., was tested three days later no change in the nature of the growth could be detected even in those parts adjoining the DH_C.

Under the experimental conditions employed, an effect was usually apparent within twenty-four hours after marginal inoculation. The first visible sign of the reaction of DH_F to the treatment, especially in small cultures, was the gradual thinning out of the compact and regular margin, fig. 13, Plate 16, into the diffuse, irregular growth characteristic of the margin of young DH_C cultures, fig. 18, Plate 16. At this stage a slight change in the rate of spread was observable in small cultures, but considerable irregularities frequently occurred. The cultures gradually assumed a regular margin typical of older DH_C, fig. 19, Plate 18, and by the time this was accomplished the rate of spread had slowed down very considerably and the zones of aerial mycelium normal to DH_F ceased to be formed. The whole culture eventually showed the characteristics of DH_C. In larger DH_F cultures the influence of DH_C advanced gradually from the inoculated margin. As the conversion was very gradual and the mycelium of the converted region spread at a rate equal to that of DH_C while the unconverted mycelium advanced at the faster rate of DH_F, the cultures, after a time, became pear-shaped. It is apparent, therefore, that the estimate of the radius of such cultures by measurement in any one direction as given in Table XI is only an approximation for the whole culture, but it is nevertheless reliable for purposes of comparison. The larger the culture at the time of inoculation with DH_C, the larger the area that remained unconverted. It is possible, however, that given long enough all such cultures would eventually change entirely into DH_C.

DH_F inoculated with DH_C at the centre.—Cultures of DH_F of different sizes were each

inoculated at the centre with young DH_C mycelium. The radii of the cultures were measured at intervals before and after the central inoculation, and the morphological characters of the growths were noted. The results are given in Table XII and selected results illustrated in fig. 7.

In general, the results of these experiments resemble those obtained from peripheral inoculation of DH_F with DH_C. The rate of spread of cultures which were of 3·0–7·5 mm. radius when inoculated at the centre with DH_C, fell rapidly to the value characteristic of DH_C ; that of larger cultures (8–10 mm. radius) was also reduced but to a less degree ; that of still larger cultures remained unchanged, Table XII and fig. 7.

Mycelium removed from various parts of small cultures (3·0–7·5 mm. radius) twenty-four hours after inoculation with DH_C, developed solely into DH_C indicating that these cultures had been converted throughout. When larger cultures (8–10 mm. radius) were tested, however, inocula from along different radii proved to be of different nature since some developed into DH_C, others into DH_F, and others again into cultures of a character intermediate between DH_C and DH_F (fig. 8). It was therefore evident that in cultures of this size inoculated at the centre with DH_C, as in similar ones inoculated at the periphery, both DH_C and DH_F were present, but it was impossible to define the limits of either strain within the culture. Very large cultures retained the external

TABLE XII.—Radius (mm.) and Nature of DH_F Cultures After Inoculation with DH_C at the Centre.

Radius when inoculated with DH_C, mm.	Number of days after inoculation with DH_C.												Character 12 days after inoculation with DH_C.
	1.	2.	3.	4.	5.	6.	7.	8.	9.	10.	11.	12.	
3·0	5·0	7·0	8·5	12·0	13·5	14·5	16·0	17·5	19·0	21·0	22·0	23·0	DH_C
3·5	5·0	7·0	9·0	11·5	13·0	14·0	16·0	18·0	19·5	21·0	22·5	24·0	,,
5·0	7·5	9·0	11·5	15·5	16·5	17·0	17·5	18·5	20·0	22·0	23·5	25·0	,,
5·5	9·0	10·0	13·0	14·5	16·0	17·0	18·0	20·0	22·0	23·0	24·0	26·0	,,
6·0	8·0	11·0	14·5	16·0	17·5	19·5	21·0	23·0	25·0	27·0	29·0	31·0	,,
6·0	8·0	12·0	14·0	17·0	19·0	20·5	22·0	24·5	27·0	29·0	31·0	33·0	,,
7·5	12·5	15·0	17·0	19·5	22·0	23·5	24·5	26·0	28·0	29·0	31·5	32·0	,,
8·5	13·5	17·5	20·0	23·5	27·5	31·5	35·0	37·5	40·5				DH_C + DH_F
9·0	13·0	15·0	17·0	23·0	25·0	28·5	32·0	36·0	40·0				,,
9·0	13·5	17·0	20·5	24·0	28·0	32·0	35·0	37·5	40·5				,,
9·5	14·5	19·0	21·5	25·0	28·5	32·0	35·0	38·0	40·5				,,
10·0	14·0	16·5	19·0	24·0	25·0	29·0	34·0						,,
11·0	15·0	17·5	20·0	25·0	27·0	32·0	36·0						DH_F
11·0	16·0	18·0	20·0	25·0	27·0	32·0	36·0						,,
15·0	19·0	22·5	28·0	33·0	36·0	40·0	45·0						,,
19·5	23·0	29·0	32·5	36·0	39·5	44·5	50·0						,,

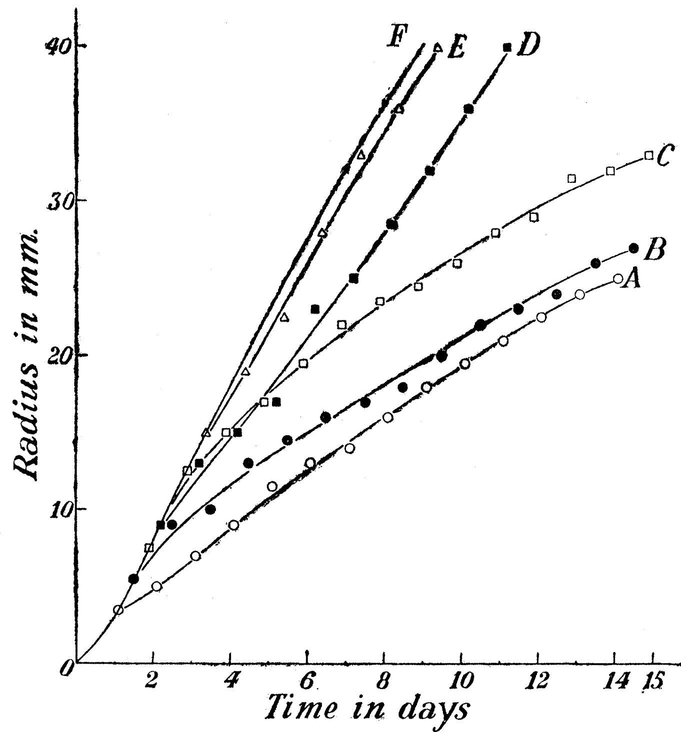

Fig. 7.—Graphs showing change in rate of spread of DH_F cultures after inoculation with DH_C mycelium at the centre. A. Culture inoculated with DH_C when of 3·5 mm. radius. B. Culture inoculated with DH_C when of 5·5 mm. radius. C. Culture inoculated with DH_C when of 7·5 mm. radius. D. Culture inoculated with DH_C when of 9·0 mm. radius. E. Culture inoculated with DH_C when of 15·0 mm. radius. F. Culture not inoculated with DH_C.

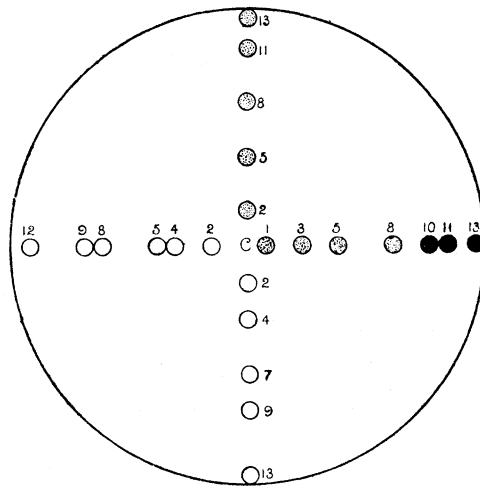

Fig. 8.—Diagram illustrating the nature of growth arising from different parts of a DH_F culture twenty-four hours after inoculation with DH_C mycelium at the centre. Radius when inoculated with DH_C mycelium, 9·0 mm.; radius when tested, 13·0 mm. Black circles, white circles, and shaded circles represent inocula which respectively developed as DH_F, DH_C, or a mixture of the two. Numerals indicate the distance in mm. from C, the DH_C inoculum. ○ Inocula developing into DH_C ; ⊛ Inocula developing into DH_C + DH_E ; ● Inocula developing into DH_F.

appearance of DH_F and when tested three days after the central inoculation were found to be pure DH_F throughout.

The first visible signs of conversion of DH_F into DH_C and the subsequent behaviour of the converted cultures were exactly similar to those described for DH_F inoculated with DH_C at the periphery; the margin lost its original regularity, gradually passed through a stage of diffused irregularity usually found in young DH_C and finally assumed the regular outline of an old DH_C culture, fig. 15, Plate 16. This applies particularly to cultures not exceeding 7·5 mm. in radius at the time of central inoculation. In larger cultures the transformation was less evident; the rate of spread was approximately that of normal DH_F, but the external character was modified. No zone of aerial mycelium was formed after the DH_C inoculation, but that already in existence persisted.

DH_F inoculated with a sector of DH_C.—In order to ascertain the extent to which an increase in the size of the DH_C inoculum may influence the reaction of DH_F, experiments were designed in which a sector removed from a DH_F culture was replaced by a similar sector from a DH_C culture, a process to which the term "grafting" is applied. All the cultures employed exceeded 10 mm. in radius, and it was observed that they were converted into DH_C more readily than were the large cultures of the previous experiments, probably because a sector is in contact with a greater area of DH_F than is a small inoculum.

One culture was thoroughly tested seven days after the grafting operation in order to determine the orientation of DH_C and DH_F within it at that stage, and to a less extent the direction of advance of the change from DH_F to DH_C. The culture at the time of grafting was three days old and 11 mm. in radius, and the area of the sector replaced by DH_C was about one-sixth that of the whole. The subsequent growth is represented diagrammatically in fig. 9, where successive lines denote the boundaries of the culture three, six, seven, eight, nine, and ten days (according to the numerals attached) after the initial inoculation with DH_C.

It should be noted that the DH_C spreads at its usual rate and the DH_F at a rate dependent upon its position in relation to DH_C. The DH_F farthest removed from the DH_C sector advanced most rapidly and that in the immediate vicinity of DH_C least quickly, hence the culture gradually assumed a pear-shaped outline (fig. 20, Plate 18).

As a preliminary to testing the mycelium, the area of the culture was mapped out by lines on the back of the plate drawn at definite distances apart and as shown in fig. 10, A.

Inocula were taken from along each of the lines XX′, AA′, BB′, etc. Each inoculum from the parallel lines was numbered according to its distance from the nearer end of the line from which it was taken, *e.g.*, inoculum "15" on the part of the line CC′ left of the vertical line XX′ originated from a point in the culture 15 mm. from C; inoculum "1" on the section of CC′ right of XX′ was taken from the margin at C′. The numbers assigned to inocula on the long axis XX′ denote the distance of each in

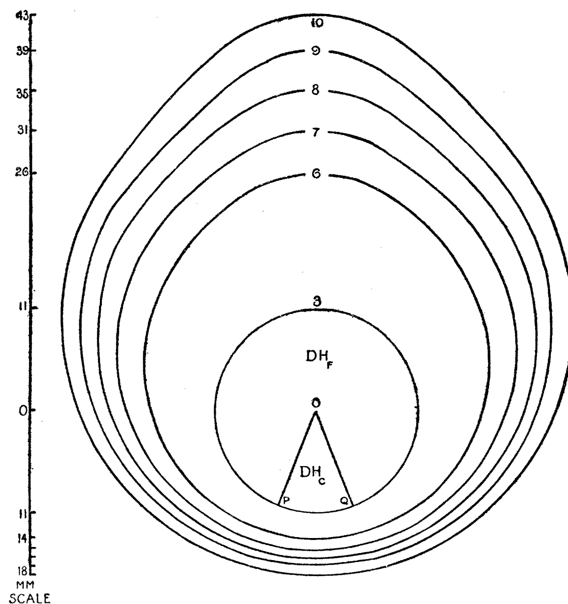

Fig. 9.—Diagram illustrating the differential rate of spread of different parts of a DH_F culture after replacement of a sector with DH_C. Each line represents the outline on a particular day (indicated by the numeral) after the initial inoculation with DH_F. OPQ = substituted sector.

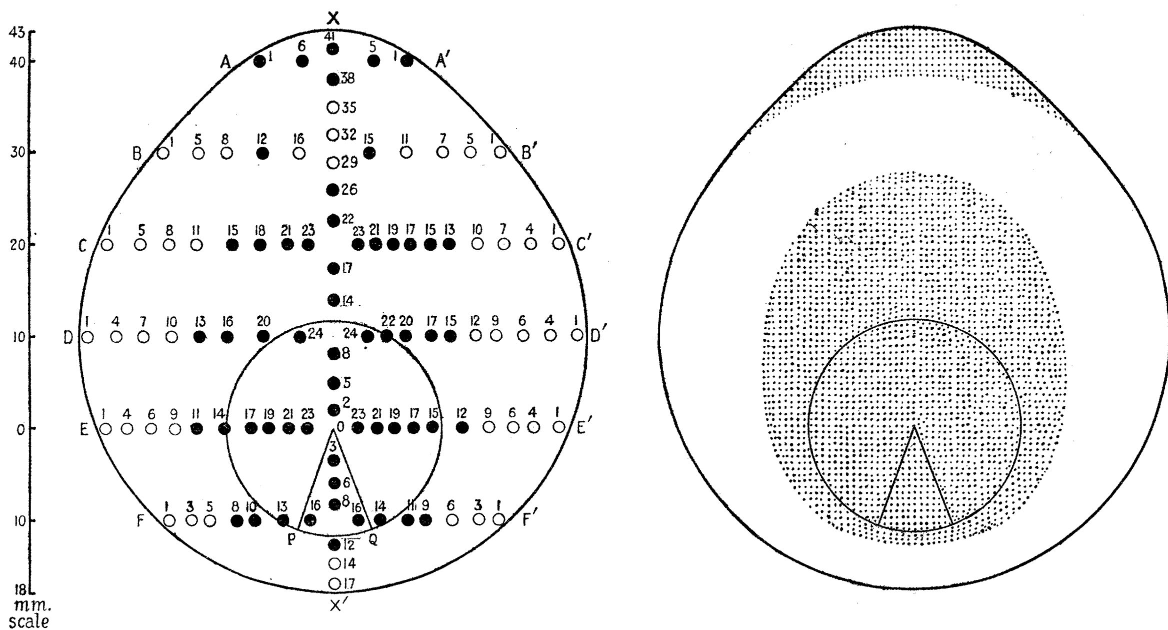

Fig. 10, A and B.—A. Diagram illustrating the mycelial test of a DH_F culture seven days after replacement of a sector with DH_C. OPQ = substituted sector. Black circles and white circles represent inocula developing as DH_F and DH_C respectively. (For further explanation, see text.) B. Diagram showing the distribution of DH_F and DH_C as determined by the mycelial test. Shaded area, DH_F; unshaded area, DH_C.

mm. from O, the point of original inoculation with DH_F. The test inocula were grown in separate plates of standard medium and the type of growth resulting from each

was noted. The distribution of the two kinds of mycelium obtained is illustrated in fig. 10, B, where the shaded and unshaded areas represent DH_F and DH_C respectively. It will be observed that DH_F occupied the central region and also a crescent-shaped area at that part of the margin farthest removed from the grafted sector of DH_C. A broad peripheral zone of DH_C encircled the DH_F area.

A second test of the mycelium from different parts of the same culture was made six days later. It was found that the proportion of DH_F in the advancing zone was by that time still further reduced and occupied a peripheral region only 2–3 mm. wide. The central area of DH_F had, however, increased showing that the part of this region which had given DH_C at the first test had now changed back into DH_F.

At the time of the second test the central DH_F was composed of mycelium having different histories, viz., (1) the original DH_F culture, (2) mycelium which developed from (1) and remained as DH_F, (3) mycelium which developed from (1), was converted into DH_C and later saltated back into DH_F, (4) the grafted DH_C sector which had saltated into DH_F, and (5) mycelium which developed from (4) as DH_C and was later converted into DH_F.

At the time of the first test the central DH_F region may have been composed only of mycelia (1), (2), (4), and (5) enumerated above, or some (3) may have been present as well.

The DH_C region in both tests must have been composed of the converted DH_F and hyphæ growing from it as DH_C, together with young DH_C developing from the grafted sector.

Experiment with old DH_C (virtual DH_F).—Since the older regions of large DH_C cultures are known to saltate into DH_F, it was decided to test the reaction, if any, of DH_F to old DH_C and of old DH_C to young DH_C.

DH_F inoculated with old DH_C.—DH_F cultures of different radius (2·5, 3·0, 4·5, 5·5, 7·5, 10·0, and 10·5 mm.) were inoculated either at one spot in the periphery or at the centre with mycelium from the oldest part of a DH_C culture of 18 mm. radius. Little effect was observed on either the growth-form or the rate of spread of the DH_F. As a control experiment DH_F cultures of various radii were inoculated at the centre or at a peripheral spot with young DH_F. No deviation from the normal growth occurred.

DH_C (large culture) inoculated at the centre with young DH_C.—Tests were made of inocula from along four radial lines starting from the centre of a DH_C culture of 18 mm. radius, and DH_F developed from all of them. An inoculum of young DH_C was then placed at the centre of the culture. Mycelium from along radial lines adjacent to those previously investigated proved, when tested, to be all DH_F. Hence it appears that once DH_F has been produced by saltation at the centre of a DH_C culture it cannot while still within the parent body be changed back by inoculation with young DH_C.

3. *Established DH_C Cultures Inoculated with DH_F.*

Cultures of DH_C of various sizes were each inoculated with young DH_F at one marginal spot. The character of the DH_C remained unchanged and any growth from the added inoculum was unrecognizable as DH_F. Further, a sector of a DH_F culture was grafted into a culture of DH_C, and again the DH_C continued to grow in a normal manner, while fresh growth from the DH_F sector was indistinguishable from the other strain in both morphological character and rate of spread. Fig. 22, Plate 18, illustrates the type of growth obtained ten days after a DH_F sector had been grafted into a DH_C culture of 15 mm. radius. Although the sector remained distinct in appearance, the nature of the growth indicated that the DH_F had been converted into DH_C.

4. *Effect of Age of DH_F Mycelium on the Progress of the Influence of DH_C.*

Previous experiments suggested that the age of DH_F mycelium has considerable effect on the progress through it of the influence of DH_C. To test this point further, DH_C was inoculated on to DH_F cultures from which various parts had been removed, thus restricting the direction in which the DH_C influence might spread.

Progress through young DH_F mycelium.—The centre was removed from each of a number of DH_F cultures of different radii, leaving in each a peripheral zone of mycelium 3–5 mm. wide. Each was then inoculated with young DH_C at one marginal spot, as shown later in fig. 11. The length of the radius before and at intervals after the peripheral inoculation and the nature of the growth were recorded and are given in Table XIII.

Comparison of Table XIII with Table XI shows that growth of DH_F cultures 6–11 mm. in radius, from which the centre had been removed, was retarded by peripheral inoculation with DH_C to a greater extent than was that of cultures of similar sizes which were left intact. The actual removal of the centre cannot account for this greater retardation since the control figures in Table XIII show that it produced no appreciable change in the daily radial increment as compared with that of a normal culture, fig. 2. Further, while only complete DH_F cultures not exceeding 7·5 mm. radius were converted throughout by peripheral inoculation with DH_C, the whole peripheral region of cultures up to 9·0 mm. in radius was changed within eight days when the centre was removed before DH_C was added. DH_C therefore appears to have a slightly greater effect upon an " annular " area of young DH_F mycelium than upon a complete culture containing both old and young hyphæ, although to reach the region of the " annular " culture farthest removed from the DH_C inoculum the influence must pass round the margin. It should also be noted in Table XIII that addition of DH_C produced less change in the larger cultures than in the smaller ones, although all the peripheral zones being of equal width must have been composed of mycelium of about the same age. This indicates a difference

in the response of the young mycelium of cultures of different ages, which is confirmed by experiments described later.

TABLE XIII.—Radius (mm.) and Nature of DH_F Cultures (Centre Removed) after Inoculation with DH_C at the Periphery.

Radius when inoculated with DH_C, mm.	Radius (mm.) at daily intervals after inoculation with DH_C.							Character 8 days after inoculation with DH_C.
	1.	2.	3.	4.	5.	6.	8.	
6·0	8·0	10·0	11·5	13·0	14·0	15·5	19·0	DH_C
6·5	8·0	10·0	11·5	13·5	15·5	17·5	21·0	,,
7·0	9·5	11·5	13·0	15·0	17·5	19·0	22·0	,,
7·5	10·0	11·5	13·0	15·0	17·5	19·5	23·0	,,
8·0	12·0	14·0	17·0	19·0	20·5	22·0	26·0	,,
9·0	11·0	14·0	18·0	23·0	25·0	29·0	34·0	DH_C
		*11·0	*15·0	*18·0	*21·0	*22·0	*25·0	(irregular)
10·0	13·5	16·0	20·5	24·0	27·0	32·0	37·0	$DH_C + DH_F$
				*22·0	*24·0	*25·0	*29·0	(irregular)
11·5	15·0	18·5	24·0	28·0	31·0	34·0	42·0	,,
		*16·0	*20·0	*22·0	*24·0	*25·0	*29·0	
12·5		17·5	20·5	25·0	30·0			,,
13·0		20·5	24·5	28·0	34·0			,,
14·5		21·5	24·5	29·5	35·0			,,
15·5		24·5	28·0	32·0	36·5			$DH_C + DH_F$
16·0		24·5	28·0	32·0	36·5			,,
Control Radius when centre removed.	No DH_C inoculum added. Radius at daily intervals after removal of centre, mm.							
10·5	14·5	19·5	23·0	27·0	30·0	35·0	37·0	DH_F

* Measurement at right angles to that immediately above.

Progress through old DH_F mycelium.—From a DH_F culture there was removed all but a diametrical strip 2–4 mm. wide. Each end acted thereafter as a source of fresh growth. An inoculum of young DH_C was placed on one end and the growths from both ends were examined at intervals. A series of DH_F cultures were tested in this manner. Similar but uninoculated strips were prepared as controls. The radius of fresh growth at the inoculated end (A) and the uninoculated end (B) was measured periodically from points " A " and " B " respectively. The values of the radius at end " B " in individual plates are given in Table XIV, together with the average value (from 9 plates) of the radius at end " A" where, as expected, DH_F was always converted into DH_C. Fig. 21, Plate 18, is a photograph of one DH_F culture four days after inoculation of the central strip, 17 mm. long, with DH_C at one end. It is clear that the newly developed mycelium from the inoculated end was of the DH_C type, while that at the uninoculated end was DH_F.

The fresh growth arising from end " B " was found to resemble DH_C in appearance and rate of growth when the DH_F culture employed did not greatly exceed 7·0 mm. in

radius at the time of inoculation with DH_C; mycelium of the age found at the centre of DH_F cultures of less than 7 mm. radius therefore seems to afford no check to advance of the DH_C influence. Fresh growths from the uninoculated ends of strips longer than 14 mm. were characteristic of DH_F even after intervals of eight days, indicating that the central, older mycelium had there impeded the progress of the DH_C influence.

TABLE XIV.—Radius (mm.) and Nature of Fresh Growths from Ends "A" and "B" of Central Strips of DH_F Cultures Inoculated with DH_C at End "A" Only.

Radius of culture employed, mm.	Length of strip, mm.	End.	Radius up to eight days after inoculation with DH_C.						Nature of growth.
			1.	2.	4.	5.	6.	8.	
5·5–13·5	11·0–27·0	A	1·5	3·7	7·2	9·6	11·6	15·6	DH_C
5·5	11·0	B	2·5	5·0	8·0	10·0	12·0	16·0	,,
6·5	13·0	B	1·5	4·0	7·0	9·5	11·0	14·0	,,
7·0	14·0	B	2·0	4·0	7·0	10·0	12·0	16·0	,,
7·5	15·0	B	4·0	9·0	19·0	22·0	28·0	35·0	DH_F
8·5	17·0	B	3·0	6·5	11·0	13·5	18·0	24·0	DH_C
11·0	22·0	B	4·0	9·0	16·0	21·0	25·0	32·0	DH_F
12·0	24·0	B	4·5	9·0	17·0	21·0	25·0	32·0	,,
13·5	27·0	B	5·0	9·0	17·5	19·0	22·5	30·0	,,
Control.	No. DH_C inoculum added.								
5·5–13·5	11·0–27·0		4·5	9·5	18·0	21·5	25·5	33·0	DH_F

A further series of similar strips of various lengths were inoculated at the centre with young DH_C. It was found from the nature of the growths at either end of the DH_F strips that DH_C had only influenced the DH_F mycelium at ends which were less than approximately 8 mm. distant from the centre.

Since the influence of DH_C is able to pass right across a DH_F culture of 14 mm. diameter through a narrow strip, but not from the centre to the periphery of a DH_F culture somewhat less than 14 mm. in radius, it appears that the age of the DH_F mycelium rather than the length of the strip determines whether or not the influence of DH_C will be apparent in the fresh growth at the ends of the strip.

5. *Rate of Progress of the Influence of DH_C.*

Reference has already been made to the complete conversion of cultures of DH_F not exceeding 7·5 mm. in radius into DH_C within about twenty-four hours after inoculation with DH_C irrespective of the position of the DH_C inoculum. The shortest distance between the DH_C inoculum at the margin of the largest of such cultures and the mycelium farthest removed from it is equal to the diameter of the culture, *i.e.*, 15·0 mm. As the influence of DH_C travels right across the culture within twenty-four hours its minimum rate of progress must be 15 mm. per day, *i.e.*, about 10·5 μ per minute.

The data in Table XIII indicate that in the largest DH_F cultures (8 mm. radius) which change completely into DH_C after removal of the centre and inoculation with DH_C, the influence travels peripherally a distance of 20–25 mm. within twenty-four hours, or at a rate of approximately $17 \cdot 5$ μ per minute.

In a similar experiment with an older DH_F culture (14 mm. radius) the central area was removed to leave a peripheral zone 4 mm. wide. This zone was inoculated with young DH_C mycelium at one marginal spot, fig. 11, A. After forty hours when the culture had attained a radius of 22 mm., mycelium round the periphery on either side of the DH_C inoculum was tested. It was found that the greatest distance to which the influence from the DH_C had spread was 30 mm. on one side of the inoculum and 26 mm. on the other, fig. 11, B. Assuming that the progress was along a straight line the minimum rate of advance of the influence must have been 26 mm. in forty hours, or about 11 μ per minute. It therefore appears that the rate of advance of the DH_C influence through the younger mycelium of old DH_F cultures is nearer to that through the older than that through the younger mycelium of young cultures.

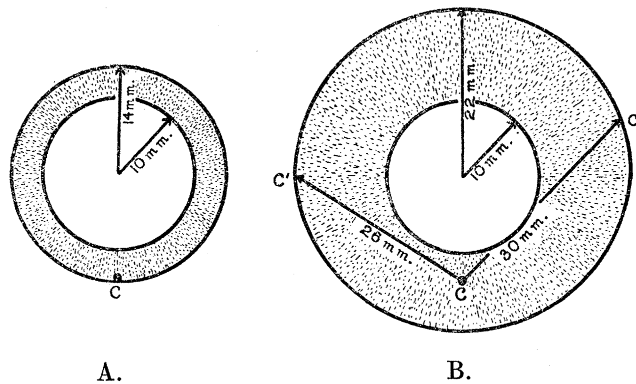

FIG. 11, A and B.—Illustration of an experiment to determine the rate of spread of the DH_C influence through the peripheral zone of a DH_F culture. Shaded area, mycelium; unshaded area, space left by removal of the centre. A. Culture when inoculated with DH_C mycelium. B. Culture forty hours later. C = DH_C inoculum; C′ and C″ = the peripheral points farthest from C, from which inocula growing as DH_C were obtained.

A much lower rate of progress was observed when half of a DH_F culture of 20 mm. radius was removed and replaced by a similar half from a DH_C culture. It was found that after five days while young hyphæ of the grafted DH_C had advanced radially only 7 mm. the influence of this strain had reached points round the periphery of the DH_F culture as far distant as 30 mm. from the junctions of the DH_F and DH_C margins. The rate of progress of the influence must therefore have been 30 mm. in one hundred and twenty hours or approximately $4 \cdot 2$ μ per minute.

In still larger cultures of DH_F (25 mm. radius) inoculated with DH_C at one peripheral spot the DH_C influence travelled through the young DH_F mycelium only 2 mm. in forty-eight hours, *i.e.*, its rate of progress was about $0 \cdot 7$ μ per minute.

VI. A Consideration of the Factors which may be Responsible for the Conversion of DH_F.

Sufficient evidence has been given to prove that under certain conditions DH_C converts DH_F into DH_C. It would seem that the change may be due (1) to the effect of soluble staling products of metabolism, or (2) to the effect of volatile products of metabolism or (3) to some effect of contact of the mycelia of the two strains. Each of these possibilities will be considered separately.

1. *Staling Products.*

500 c.c. of liquid standard medium (without agar) was divided equally between twenty-five 100 c.c. flasks which were then autoclaved and inoculated with DH_C. After fourteen days the staled medium from twelve of the flasks was filtered through very fine muslin and used for making up media A, B, and C, the compositions of which are shown below :—

$A_{(14)}$ 1·5 gm. agar dissolved in 20 c.c. water and added to 80 c.c. staled medium.
$B_{(14)}$ 0·9 gm. agar dissolved in 20 c.c. water and added to 40 c.c. staled medium.
$C_{(14)}$ 1·2 gm. agar dissolved in 40 c.c. water and added to 40 c.c. staled medium.

The mixtures were alike in the proportion of agar present (1·5 per cent.), but differed in the percentage of staling products. Since autoclaving may alter the nature of staling substances, the agar was mixed with the requisite quantity of water, autoclaved separately and then added to the staled medium which, to ensure homogeneity in the mixture, had been slightly warmed. Four plates were made from each member of the series and each plate was inoculated with DH_F at the centre.

A similar series, $A_{(28)}$, $B_{(28)}$, and $C_{(28)}$, was prepared using the medium from the remaining thirteen cultures fourteen days later. Each plate, as before, was inoculated with DH_F at the centre.

The radius of each culture was measured periodically and the results are given in Table XV.

It will be seen from Table XV that the presence of staling products of the growth of DH_C appreciably arrested the spread of DH_F. In the first series spread is reduced by nearly one-half that observed in agar, but is little affected by degree of dilution of staling products. In the second series where more concentrated staling products were used the reduction of spread is very considerable and dilution has a marked effect. The nature of the mycelium in all these cultures proved on test to be DH_F.

Similar results were obtained when DH_F was grown on agar mixed with medium staled by previous growth of the same strain.

In order to ascertain the reaction of DH_F to the combined staling products of DH_C and DH_F under conditions in which lack of nutrition could play no part the following experiments were made: One hundred conical flasks, each containing 20 c.c. liquid

TABLE XV.—Radius (mm.) of DH_F Cultures Grown on Agar Mixed with Various Proportions of Staled Medium from DH_C Cultures. (For actual composition of media, see p. 148.)

Medium.	Number of days after inoculation.			
	2.	4.	6.	9.
Control (agar)	5·0	11·0	16·0	26·0
$A_{(14)}$	5·0	11·0	14·0	15·5
$B_{(14)}$	5·0	10·0	13·0	15·0
$C_{(14)}$	4·0	10·0	13·0	14·5
$A_{(28)}$	0·0	1·0	1·5	2·5
$B_{(28)}$	0·5	1·5	2·0	3·0
$C_{(28)}$	1·5	4·0	4·5	5·0

standard medium, were inoculated with DH_C and a similar hundred with DH_F. After twenty-eight days the staled medium from the two sets of cultures was strained off separately into two different flasks and samples from each were mixed in the various proportions shown in Table XVI. The p_H of the resulting solutions ranged from 7·8 in the one containing no DH_C products, to 8·3 in the one containing no DH_F products. 80 c.c. of each mixture was made up to 100 c.c. by addition of 20 c.c. sterilized standard medium of five times the normal strength, so that the final media, disregarding any unused ingredients in the staled solution, were equal in concentration to normal standard medium and contained staled medium in a total proportion corresponding to that of " A " in the previous experiment. Four plates were prepared from each member of the series and inoculated with DH_F at the centre. The radius of growth was measured periodically and the values obtained are given in Table XVI.

TABLE XVI.—Radius (mm.) of DH_F Cultures Grown in Mixtures of Staled Media from DH_C and DH_F Cultures.

% staled solution in final medium.		Number of days after inoculation.				
DH_C.	DH_F.	2.	4.	5.	7.	9.
0	0	8·0	17·0	21·0	31·0	42·5
0	80	3·5	14·5	19·0	25·0	30·0
10	70	6·5	14·5	19·5	25·0	30·0
20	60	6·5	15·0	20·0	26·5	31·5
27	53	—	—	—	—	—
40	40	5·5	13·5	19·5	25·5	30·5
53	27	4·5	12·0	17·0	22·5	29·0
60	20	5·5	13·0	18·0	24·5	30·0
70	10	6·0	13·0	17·5	22·0	26·5
80	0	4·5	12·5	17·5	23·5	29·5

Although fungal spread was considerably retarded by the presence of staled media (Table XVI), the cultures had every appearance of pure DH_F and mycelial tests proved

that no conversion had occurred. The rate of spread was about the same in all the media indicating that the relative proportions of the metabolic products of DH_C and of DH_F in no way influenced the reaction of DH_F.

2. *Volatile Products.*

The method employed was an adaptation of that described by Brown (1923). A dish of standard medium freshly inoculated with DH_F was inverted, after removal of the lid, over the bottom half of a plate of the same size containing a 5·0 mm. layer of medium inoculated at the centre with DH_C. Other newly-prepared DH_F cultures were similarly placed over cultures of DH_C which were 4, 9, 15, 18, 28, or 52 days old, over DH_F cultures of corresponding ages and over control plates of uninoculated medium. In this way DH_F was exposed either to the combined effects of the volatile products of its own activity and that of DH_C, or to the effects of increased quantities of its own products alone. The plates were kept at laboratory temperature. The radius of spread of the upper DH_F culture was measured at intervals with as little disturbance as possible, but some change in the composition of the enclosed atmosphere was inevitable with the handling of the plates. The average values obtained after eight days' growth are given in Table XVII.

Table XVII.—Radius of Eight Days' Old DH_F Cultures Inverted Immediately After Inoculation Over Cultures of DH_C or DH_F of Various Ages.

Arrangement of cultures.	Age of lower culture when placed in position, days.					
	0.	4.	9.	15.	28.	52.
	Radius of upper DH_F culture, mm.					
DH_F over DH_C ...	31·0	27·0	27·0	20·5	18·0	19·5
DH_F over DH_F ...	30·0	20·0	26·5	24·0	20·0	19·5
DH_F over uninoculated medium (control)	32·0					

It will be observed from Table XVII that (1) the volatile substances considerably retarded the spread of DH_F and (2) the growth of DH_F was as much affected by the volatile products of another DH_F culture as by those of DH_C mixed with DH_F products. Some of the DH_F cultures developed none of the aerial mycelium characteristic of this strain and appeared to some extent like DH_C in external character. Nevertheless, when mycelium from different parts was tested, typical DH_F cultures developed. It therefore appears impossible to regard the volatile substances produced after addition of a DH_C inoculum to a DH_F culture as responsible for the conversion of the DH_F.

3. *Contact of Hyphæ.*

As conversion of DH_F into DH_C could not be ascribed to the effects of the metabolic products of DH_C, it was clear that the effect of actual contact of the hyphæ should be closely investigated. An experiment was therefore devised in which from each of a number of standard-medium plates there was removed from the centre a strip of medium extending about 3 cm. in length on either side of the centre and having a width of 0·5, 1·0, 2·0, 4·0, 6·0, 10·0, or 15·0 mm. One inoculum of DH_C and one of DH_F were placed respectively on opposite sides of the gap at the centre of its upper, longer edge. It was thus possible to observe the contact of the two mycelia as the strains advanced across the gap and also the response of DH_F to the contact. Plates were prepared in triplicate and kept at 20° C. The time required for the two strains to meet was noted; the DH_F mycelium was tested before and after contact with DH_C had been established and its character was periodically recorded. It was observed that (1) growths from DH_C and DH_F inocula divided by gaps of 0·5, 1·0, or 2·0 mm. met within two days and the DH_F was speedily converted into DH_C; (2) growths from DH_C and DH_F inocula divided by gaps of 4 and 6 mm. met within three and four days respectively and both cultures of DH_F were changed entirely into DH_C within seven days after inoculation; (3) DH_F growing across a 10·0 mm. gap met DH_C on the seventh day, but was unchanged three days later; (4) growths from inocula of DH_C and DH_F separated by a 15 mm. gap failed to meet within ten days, probably on account of the lack of nutriment in the empty space, and DH_F remained unchanged.

A photograph of a number of these cultures six days after inoculation is given in fig. 23, Plate 19, in which it may be seen that conversion of DH_F into DH_C had by this time occurred in cultures where the gap was 0·5, 1·0, 2·0, 4·0, or 6·0 mm., but not in those where the gap was 10·0 or 15·0 mm. wide.

It was apparent from this experiment that close hyphal relationship of DH_C with DH_F is essential for the conversion of a DH_F culture into DH_C, and further evidence in support of this was sought by another method. The bottom half of a small petri-dish (5 cm. diameter and 2·5 cm. deep) was placed centrally within a larger and deeper dish. Sterilized medium was then poured to the depth of 0·5 cm. into the inner and to the same level in the outer dish thereby leaving a glass partition 2·0 cm. high above the surface of the medium in each plate. One inoculum of DH_F and one of DH_C were placed opposite each other on the surface of the inner and outer medium respectively and in contact with the glass partition. The arrangement is illustrated in fig. 12. Conditions were thus attained in which the two

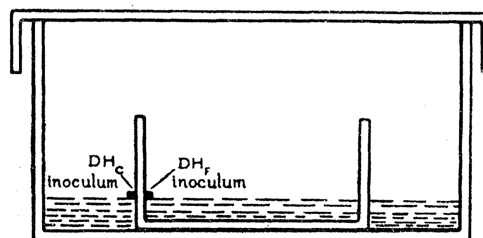

Fig. 12.—Diagram of the arrangement employed to determine the effect of DH_C on DH_F when in close proximity to it, but unable to establish contact. (For further explanation, see text.)

strains while only about 2 mm. apart (the width of the glass) could grow independently of each other's mycelium and staling products and, in fact, of all but the volatile products to which both were equally exposed. As a control DH_F was grown alone in an inner dish surrounded by uninoculated medium. The radius of each DH_F culture was measured periodically and the values obtained are given in Table XVIII.

TABLE XVIII.—Radius (mm.) of Growth from a DH_F Inoculum Separated from DH_C by a Glass Partition 2mm. thick, and of a DH_F Control (average of four plates).

Culture.	Time in days.			
	2.	4.	6.	8.
Experimental	7·0	13·5	19·5	28·5
Control	7·0	13·0	18·0	28·0

It will be seen from Table XVIII that the rate of spread of the experimental cultures was equal to that of the controls. This result indicates that the influence of DH_C on DH_F must operate either through direct contact of its mycelium with that of DH_F or through its staling products. The latter have, however, already been shown to have little effect on the conversion of DH_F.

Fusion of hyphæ.—An attempt was made to determine whether the fusion of the hyphæ of DH_C and DH_F occurs. Slide cultures with a thick or thin layer of medium inoculated with DH_C at one end and with DH_F at the other were first tried, but proved unsuitable for accurate observations in the region of contact of the two strains, on account of the abundance of hyphæ. The following slightly modified method was therefore adopted. The medium on a slide was divided into three parts by two incisions parallel to the short axis; the middle part was removed and the section on one side was inoculated with DH_C and that on the other with DH_F. As the strains advanced along the glass surface across the gap it was found on several occasions that the mycelium of DH_C became united with that of DH_F. A microphotograph showing such union is given in fig. 24, Plate 19. It should be remembered, however, that the available food material on the glass surface was negligible.

In order to ascertain if the rapid spread of the conversion could be at all connected with the movement of the protoplasm in DH_F hyphæ the following experiment was made: Slides thinly spread with standard medium were inoculated with DH_F and kept at laboratory temperature. After 2–3 days the mycelial growths were examined under high magnification. Movement of protoplasm was not found to be of general occurrence. On occasions, however, protoplasmic contents would be seen moving in mass through a number of consecutive segments of a young hypha in the direction of spread and no "back-flow" was observed. Further, some large "vacuolar" bodies contained in the protoplasm appeared to be squeezed as they passed through the

partition walls, indicating that the walls are at least partially dissolved. These observations correspond with those of TERNETZ (1900) on *Ascophanus carneus*, who termed the phenomenon "streaming," and those of POTEBNIA (1907) on *Sphaeropsis pseudo-diplodia* and other ascomycetes.

A number of observations were made on the movement of a single "vacuole," which was followed for a distance of 2–3 mm. in a hypha which included a number of partitions. The rate of streaming worked out at about 2·3 mm. and in some cases as high as 9·2 mm. per minute. Considerable further work is necessary before the significance of this phenomenon in connection with the conversion can be definitely settled.

VII. DISCUSSION.

The experiments described in the foregoing pages reveal several interesting features in the relationship between the two strains, DH_C and DH_F, of *Diaporthe perniciosa.*

Conclusive proof has been obtained that mycelium from any part of a typical DH_C culture may grow as DH_F. Furthermore, there is nothing in the external character of such cultures to suggest that a mixture of strains is present. It was known (HORNE and DAS GUPTA, 1929) that, on attaining a certain age, DH_C mycelium, if transferred to fresh medium, develops as DH_F, but the existence of DH_F potentialities in young DH_C was unrecognizable so long as inocula of the customary size (approximately 1 cu. mm.) only were tested. When, however, minute fragments of mycelium of any age were individually used as inocula or were, after suspension in water, added to melted medium or sprayed on to the surface of solid medium, an extraordinarily high proportion grew as DH_F. The proportion of DH_F cultures obtained in different experiments varied with the age of both the mycelium itself and of the parent culture, *e.g.*, the relative number of fragments giving this strain was higher in both old and young parts of an old culture than in the corresponding parts of a young culture (see Tables I, II, and IV) and higher in the older than in the younger region of an individual culture (see Table III). It is particularly worthy of note that in all tests, except those of very young cultures no more than 1·5 mm. in radius, the DH_F colonies produced far outnumbered the DH_C. Further, although no fragments of extremely young cultures (0·5 mm. radius) grew as pure DH_F, some developed into cultures which appeared to be mixtures of DH_C and DH_F, indicating that even the youngest DH_C mycelium is not entirely free from DH_F characters.

Since the majority of fragments of extremely young DH_C mycelium succeeded in reproducing the parent culture, the appearance of DH_F from other fragments cannot be attributed to any change attendant upon the actual operation of cutting up the inoculum. It may therefore be assumed that the results of the "plating" experiments are a fair indication of the true mycelial character at different parts of a culture and hence that an apparently pure DH_C culture is in reality an intimate mixture of DH_C with DH_F.

The manner of distribution of the two strains within one culture remains, however, obscure. Observations on the nature of the growth from micro-dissected fragments of single hyphæ (see fig. 3) showed that DH_C and DH_F may develop respectively from two branches of the same hypha or even from successive portions of one branch. Hence it appears that in a DH_C culture the characters of both strains may simultaneously exist within an individual hypha.

The tips of hyphæ in the advancing region of a DH_C culture were found to be of two types, one thick and one thin as described in earlier pages, and the growths from them (with the exception of one mixture) were of two kinds, DH_C or DH_F. An attempt to correlate these facts was unsuccessful, since DH_C developed from few of the hyphal tips, DH_F grew from both kinds of tip and many of both types of hyphal tip disintegrated without developing. As explained before, disintegration can be ascribed neither to the smallness of the tips nor to the absence of a nucleus. The potentialities of the hyphæ which disintegrated are entirely a matter of conjecture, but it is interesting to note that even if all of them were potential DH_C (which is most unlikely, judging from the morphological resemblance of these tips to those that grew as DH_F), the maximum possible number of tips $(2 + 16)$ producing DH_C would still have been less than the number (26) which gave DH_F.

Although in these micro-dissection experiments the nature of growth of a tip could be no more correlated with its length than with its morphological character (see fig. 4), there is evidence that the probability of a particular mycelial fragment growing as DH_F is not entirely unrelated to its size, since the proportion of DH_F cultures obtained was less in experiments where large inocula were crushed into smaller pieces and in those where inocula were about 0·5 cu. mm. in size, than in the micro-dissection experiments where each inoculum contained only one fragment of a hypha. In conjunction with these facts it should be recalled that the presence of DH_C character inhibits the expression of DH_F character, and that cutting apparently does not destroy the power of a fragment to grow as DH_C. It therefore appears possible that the properties which are essential to a mycelium if it is to appear as DH_C may not be continuous throughout the mycelium, but rather may be localized at various points in individual hyphæ, and hence the smaller the fragment the greater its chance of being free from properties of DH_C and therefore of growing as DH_F.

The transformation of DH_F into DH_C by DH_C hyphæ may be regarded as another aspect of the phenomenon of conversion, and shows that DH_C mycelium has as great an influence on separate cultures of DH_F as it has on the DH_F hyphæ occurring within its own body. The occurrence of the conversion cannot be questioned; addition of a DH_C inoculum to a DH_F inoculum or to an established DH_F culture led to the formation of growths which were identical with a typical DH_C culture in rate of spread, in external characters, and in the nature of subcultures from inocula of the usual size.

An almost incredibly small proportion of DH_C is sufficient to cause conversion. Every inoculum composed of DH_C mixed with, or in contact with, two, four, or even six times

its bulk of DH_F, developed as typical DH_C (see Tables VII and IX), and further, under the influence of a 1 mm. cube of DH_C, cultures of DH_F with radius up to 7·5 mm. completely changed into DH_C within twenty-four hours, and cultures as much as 10–18 mm. in radius were appreciably modified (see Tables XI and XII).

The age of the fungal growths concerned markedly affects the amount and rate of conversion. An inoculum of old DH_C is unable to change DH_F, most probably because it is itself virtually DH_F. That part of an old DH_C culture which has acquired the character of DH_F cannot be changed into DH_C by addition of young DH_C. Inocula of old DH_C, however, in the presence of young mycelium of this strain, develop as DH_C and not as DH_F (see Tables VIII and X). A young DH_F culture is more sensitive to DH_C than is an old culture, *e.g.*, peripheral inoculation with DH_C of a DH_F culture of 7·5 mm. radius was followed by rapid conversion of the whole culture, while similar treatment of a DH_F culture 32 mm. in radius led to no detectable change within three days, even in the DH_F mycelium adjoining the DH_C inoculum, although this mycelium could have been no older than that at the periphery of the younger culture. Young DH_F is more readily converted than is the older mycelium in cultures of the same age. For example, DH_F cultures of radius 11–18 mm., inoculated at the margin where the mycelium was young, were changed into DH_C within twelve days in the parts near the inoculum (see Table XI), but cultures of the same dimensions inoculated with DH_C at the older, central region were unaltered after an equal period of time (see Table XII). The annular and the central strip experiments also support the view that the age of a DH_F growth may determine both the extent and the promptness of its response to the presence of DH_C, and also show that the spread of the DH_C influence is quite independent of the direction of growth of DH_F.

There is some evidence that the stability of a DH_F culture may appreciably increase at the time when the radius is about 8 mm. The DH_C influence readily passes from one end to the other of a diametrical strip of a DH_F culture of about 8 mm. radius, but not through a similar strip of a slightly larger culture. Also, as mentioned before, all the complete cultures not exceeding 7·5 mm. in radius inoculated with DH_C at either the margin or the centre were entirely converted within twenty-four hours, while those a little larger were still incompletely changed twelve days after addition of DH_C.

The rate of progress of the DH_C influence through the DH_F mycelium, as determined from the data obtained in the various experiments under discussion, may have any value from 0·7 μ or less per minute through very large and therefore old cultures up to 17·5 μ per minute through young parts of young cultures. Calculations from data obtained from the "annular" experiments showed that the DH_C influence passes from the periphery of a young culture (8 mm. radius) at the rate of at least 17·5 μ per minute, but round the equally young periphery of the older culture (14 mm. radius) at the rate of 11·0 μ per minute, thus confirming the suggestion that the age of a DH_F culture influences the response of its youngest mycelium to the addition of DH_C, although the young hyphæ of all cultures are similar in appearance.

The reversion to DH_F of mycelium which had been previously converted from DH_F to DH_C is of particular interest. It shows that the effect of increasing age is the same in a converted DH_F as in a typical DH_C culture. Hence there seems to be no physiological difference whatsoever between such cultures and it is reasonable to suppose from this that a typical DH_C culture contains throughout its whole existence a large element of DH_F such as is certainly present in DH_F converted to DH_C. This is precisely the conclusion suggested by all the earlier experiments with DH_C alone, and the results of the two lines of investigation support each other.

Having established the fact of conversion, it is of interest to review the means by which DH_C may exert its influence on DH_F.

An enquiry into the possible role of the non-volatile staling products of the metabolism of DH_C showed that in the presence of increasing concentrations of these substances the rate of spread of a DH_F culture is reduced, but the nature of the mycelium is unchanged; further, the reaction of DH_F in all media containing equal concentrations of staling substances was the same whether these were entirely products of DH_C or of DH_F, or were a mixture of the two (see Tables XV and XVI). It is therefore concluded that non-volatile staling products can be of no great importance in conversion.

Volatile products of growth were found to have much the same effect on DH_F as have the non-volatile substances. The spread of a DH_F culture exposed to increasing concentrations of volatile products was retarded whether the latter emanated from DH_C and DH_F or from DH_F alone; the mycelium assumed some of the characteristics of DH_C, but proved on subculture to be pure DH_F. Other experiments in which DH_C and DH_F were grown in the same plate showed that altering the distance between the strains, although in no way able to affect the action of volatile products, influences the conversion of DH_F. Hence it appears unlikely that the action of volatile substances is responsible for the conversion of DH_F.

A more profitable line of investigation was that of the relation between conversion of DH_F and contact of the DH_F hyphæ with those of DH_C. The experimental results have been dealt with at some length in earlier pages and indicate that however near a DH_F culture may be to one of DH_C it will not be converted into DH_C unless and until the two strains meet. This should not be taken to mean that DH_C must intermingle with DH_F throughout the whole converted area. Such a conclusion is untenable, since a very small DH_C inoculum suffices to convert rapidly a large bulk of DH_F while the normal rate of spread of DH_C is only 2 mm. per day or about $1 \cdot 4$ μ per minute. Taking three times this value, to make ample allowance for the more rapid growth of the few hyphæ which are always found ahead of the rest, the rate of spread is very low compared with the maximum rate of advance ($17 \cdot 5$ μ per minute) of the conversion. It cannot be argued that DH_C may be stimulated to grow more quickly within a DH_F culture, since it is most unlikely that conditions which have been proved to reduce the rate of spread of DH_F to a value no higher than that normal for DH_C should at the same time activate DH_C to spread at a rate exceeding the normal.

It is highly improbable that mere contact of DH_C with DH_F hyphæ promotes conversion of the latter. Contact is no doubt the essential preliminary to a fusion which initiates conversion. Such fusions though not demonstrated in normal cultures have been proved to occur under certain experimental conditions.

As far as the author is aware, nothing parallel to this conversion of one strain of *Diaporthe perniciosa* into another has been recorded for any other Ascomycete. The only phenomenon at all comparable with it is the conversion of a haploid mycelium of the basidiomycete, *Coprinus lagopus*, into the diploid state in the presence of diploid hyphæ of the same fungus, as described by BULLER (1931). In both processes a minute fragment of one mycelium is sufficient to change a large growth of the other; the converted mycelium assumes the character of the one responsible for the change; the converting influence spreads very quickly and at approximately equal rates in both (20·0μ per minute in *Coprinus lagopus* and 17·5 μ per minute through young DH_F, if the course taken is assumed to be straight); and young mycelium responds more readily than the old. These similarities in experimental facts suggest a correspondence in the mechanism of the two conversions. BULLER (1931) puts forward the theory, partly based on the results of LEHFELDT'S (1923) cytological work with *Typhula erythropus*, that when a diploid mycelium of *Coprinus lagopus* comes in contact with a haploid, fusion occurs and a nucleus of the opposite sex from that of the haploid nuclei passes from the diploid to the haploid, becomes associated with a nucleus of the latter and thus " diploidizes " one cell. The " stranger " nucleus then divides. One daughter nucleus travels through the hypha, enters the next cell and " diploidizes " that. The process is repeated in all directions and from all points of fusion until the former haploid mycelium has become diploid throughout.

The mechanism by which fusion between the hyphæ of the two strains of *Diaporthe perniciosa* leads to conversion of DH_F to DH_C is obscure. It may be the passage of a nucleus from DH_C to DH_F and the spread of the products of the division of the nucleus through DH_F, as in diploidization of *Coprinus lagopus*. It must be remembered, however, that in Ascomycetes a vegetative diploid mycelium is, as yet, unknown, and the relationship between the two strains is hardly compatible with the view that DH_C is diploid and that DH_C diploidizes the haploid DH_F mycelium.

On the other hand, it is possible that the whole mechanism is cytoplasmic. If this is so, there are strong indications that the property which enables DH_C to convert DH_F must be at localized points in the cytoplasm, since it has been shown that a converted DH_F culture is constitutionally identical with a typical DH_C culture and that two contiguous fragments of one hypha of an ordinary DH_C culture may grow respectively as DH_C and DH_F.

Whether the seat of the influence is nucleus or cytoplasm, the extremely rapid spread of conversion may possibly be due to the transport of the " converting substance " along with a rapidly moving stream of protoplasm. The occurrence of unidirectional mass movement of protoplasm has been demonstrated in certain hyphæ of young DH_F.

It has also been shown that the partition walls of a relatively young hypha are partially dissolved and allow almost free movement of protoplasm in mass from cell to cell. Such protoplasmic "streaming" has been observed by POTEBNIA (1907) in *Sphæropsis pseudodiplodia* and *Diplodia melanea*, by TERNETZ (1900) in *Ascophanus carneus*, and by REINHARDT (1892) in some species of *Sclerotinia*. According to POTEBNIA, where the movement of protoplasm is rapid the "back-flow" occurs but seldom. How such unidirectional movement of protoplasm in mass can continue without accumulation at one end and reduction at the other does not seem yet to be explained. The connection of this phenomenon with the spread of conversion is obscure, and much further work is necessary to elucidate the problem.

In view of the converting influence of DH_C the saltation of DH_C in the older part of a culture into DH_F is of peculiar interest. Presumably the factors responsible for the conversion when a comparatively young mycelium of DH_C is in contact with DH_F mycelium fail to operate within the body of a DH_C culture. Possibly this is because the hyphæ which have changed into DH_F from DH_C are more resistant to further change. In this connection it may be useful to remember that DH_C is able to convert little or none of the mycelium (even in the advancing region) of a large DH_F culture. It is also possible that the protoplasm of the mycelium which has saltated into DH_F is no longer continuous with the unchanged mycelium of DH_C and that no fresh fusion can occur between the changed and unchanged mycelium in the body of a DH_C culture. This view finds some support in the work of POTEBNIA (1907), who investigated the protoplasmic movement in twenty-four species of Ascomycetes. In *Sphæropsis* and *Diplodia* he found that while in young hyphæ the velocity of protoplasmic movement was between 500 μ and 1500 μ per minute and the cross-walls apparently perforated, "Im alten Mycelium mit umbeweglichen Plasma hängen die Zellen mittelst enger Konnexionen zusammen weil die Scheidewände fast geschlossen sind."

I wish to express my thanks to Professor V. H. BLACKMAN and Dr. A. S. HORNE, for their helpful suggestions and criticism. I am indebted to Mr. H. TOOLEY for taking the photographs.

VIII. SUMMARY.

A study has been made of the remarkable relationship between the two strains of *Diaporthe perniciosa* DH_C and DH_F, and an account is given here of a series of experiments designed to elucidate this relationship.

Two distinct lines of investigation were followed, viz., (1) analysis of DH_C cultures by observing the type of growth produced by inocula, large and small, and by those consisting of a single hyphal fragment taken from different parts of DH_C cultures of various ages, and (2) examination of the effects of inoculating DH_F mycelium with DH_C.

On the standard medium used the strains are quite distinct. Cultures of DH_C have a diffuse, irregular margin and spread relatively slowly; those of DH_F are compact and spread relatively fast.

Large inocula (about 1 cu. mm. in size) of *relatively young* mycelium of DH_C cultures of *any size* developed as DH_C. Similar inocula from *older* parts of DH_C cultures of radius more than 15 mm. grew as DH_F. This phenomenon was described in an earlier paper as saltation (HORNE and DAS GUPTA, 1929). DH_C appears to have become slightly more stable than before, since DH_F does not now develop from large inocula of DH_C cultures except from mycelium older than that from which this transformation was first obtained.

A large proportion of *minute* inocula taken from *all parts* of a comparatively young DH_C culture developed as DH_F; relatively few grew as DH_C or as mixtures of DH_C and DH_F. DH_F was more frequently obtained from old than from young parts of an individual culture of DH_C, and more from old and young parts of an old culture than from the corresponding regions of a young one.

Very small fragments of hyphæ (branched or unbranched) obtained by micro-dissection from very young DH_C cultures nearly all developed as DH_F; very few as DH_C. It was nevertheless established that DH_C and DH_F may develop respectively from two branches of the same hypha, or even from successive portions of one branch. The morphological characters of a hyphal fragment of DH_C give no clue as to which type of culture it will produce. It is concluded that a DH_C culture contains the "properties" of both DH_C and DH_F, and as judged by the product of inocula the influence of DH_C on any given part of the culture decreases with time, *i.e.*, with age.

It would seem that the "properties" of the two strains are distributed generally throughout a DH_C mycelium and are present together in a single hypha. The "properties" may, however, be spacially separated in the hypha since the smaller the hyphal fragment used as inoculum the greater is the chance of DH_F appearing.

A young DH_C mycelium is able to convert a culture of DH_F into DH_C, for all inocula composed of DH_C mixed with, or in contact with, an equal or greater proportion of DH_F develop as DH_C. Established DH_F cultures, not exceeding 7·5 mm. in radius, inoculated with a small fragment of DH_C at the centre or at the periphery, assume the rate of spread and the external appearance of DH_C; larger cultures treated in the same way are converted only in part. The outstanding features of the conversion are (1) it can be effected by a relatively minute inoculum of DH_C, (2) it spreads through DH_F mycelium at a rate up to 17·5 μ per minute, and (3) it is more readily brought about in young than in old DH_F mycelium.

The possible factors responsible for the conversion are discussed. Neither volatile nor non-volatile products of the metabolism of DH_C appear to play any part in the conversion. Actual contact, however, between some, if only a few, of the hyphæ of one strain with those of the other seems to be an essential preliminary to conversion. Fusion between the hyphæ of the two strains was observed under certain conditions

and it is suggested that it is as a result of fusion that DH_C is able to exert its influence on DH_F.

The nature of the " property " by which DH_C, a slow-growing strain, dominates the faster-growing DH_F is unknown. It may be either an attribute of the cytoplasm or of the nucleus.

The rapid spread of conversion may possibly be related to a rapid protoplasmic streaming which was sometimes observed. Much further work is, however, necessary to establish a connection between the two phenomena.

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X. DESCRIPTION OF PLATE FIGURES.

Fig. 13.—DH_F. Eleven days' growth. $\times \frac{2}{3}$.

Fig. 14.—DH_C. Eleven days' growth. $\times \frac{2}{3}$.

Fig. 15.—DH_C. Thirty-one days' growth. $\times \frac{2}{3}$.

Fig. 16.—Microphotograph of diffuse, irregular margin of a young DH_C culture. $\times$ 500.

Fig. 17.—Microphotograph of compact, regular, margin of a DH_F culture. $\times$ 500.

Fig. 18.—Growth character of a DH_F culture three days after inoculation with young DH_C mycelium (c) at the periphery. Radius when inoculated with DH_C mycelium 4·0 mm. Radius when photographed, 11·0 mm. Note the diffuse, irregular margin characteristic of young DH_C cultures. $\times \frac{3}{2}$.

Fig. 19.—The same culture as in fig. 18, photographed nine days later. Radius, 23·0 mm. Note the regular margin characteristic of comparatively older DH_C cultures. $\times \frac{3}{2}$.

Fig. 20.—Growth character of a DH_F culture seven days after a sector had been removed and replaced by a similar sector of DH_C (c.s.). Radius at the time of the operation, 11·0 mm. Note the pear-shaped outline indicative of unequal growth in different directions ; the DH_C character of a large proportion of the margin ; and the presence of DH_F at the extreme top. $\times \frac{4}{3}$.

Fig. 21.—Illustration of the method employed to investigate the passage of the DH_C influence through the older parts of a DH_F culture. AB is a central strip of a DH_F culture of diameter 26 mm., inoculated with young DH_C mycelium at A. The photograph was taken three days after the operation. Note that the growth at end A is like DH_C, and that at end B (unlike that at the corresponding point in a shorter strip) is like DH_F. $\times \frac{3}{5}$.

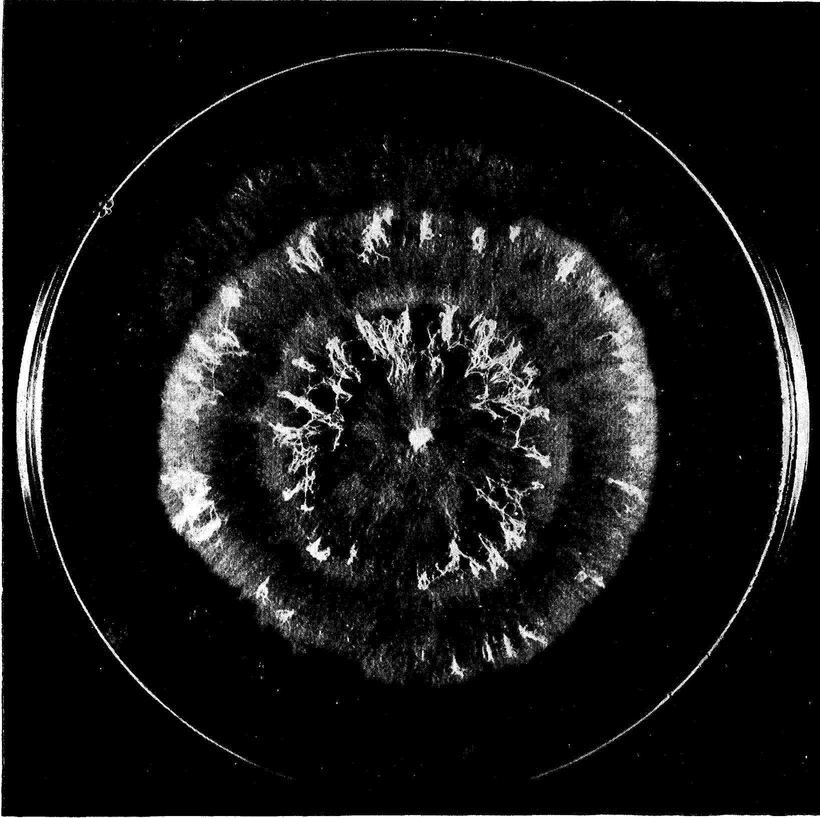
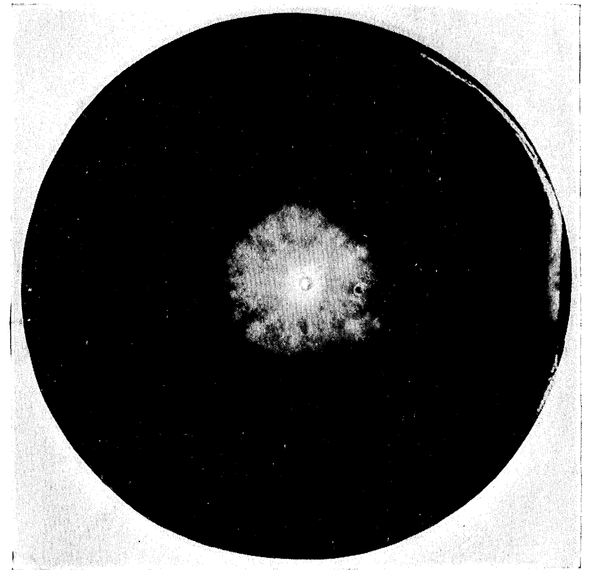


Fig. 13.



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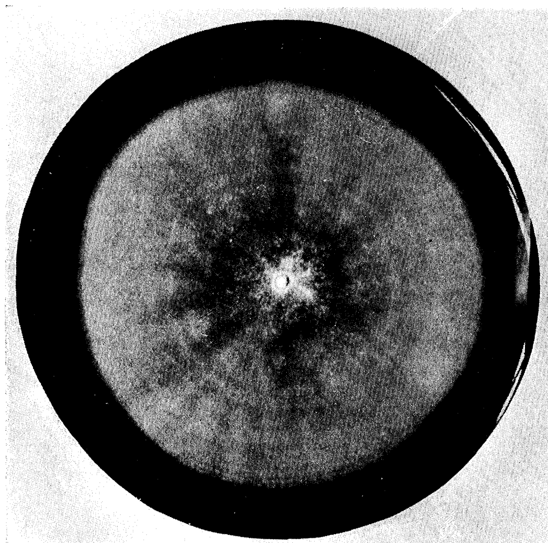

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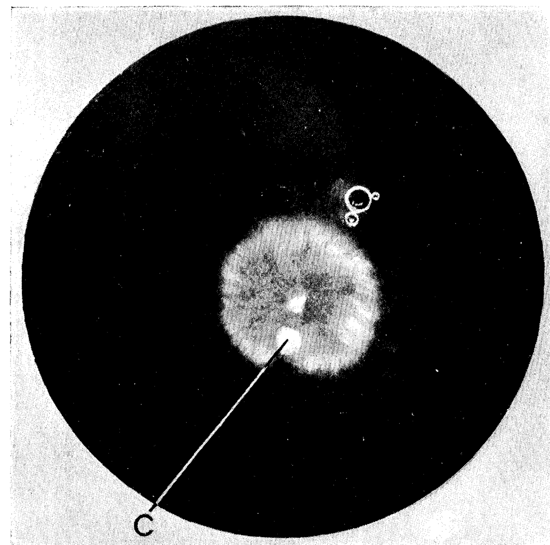

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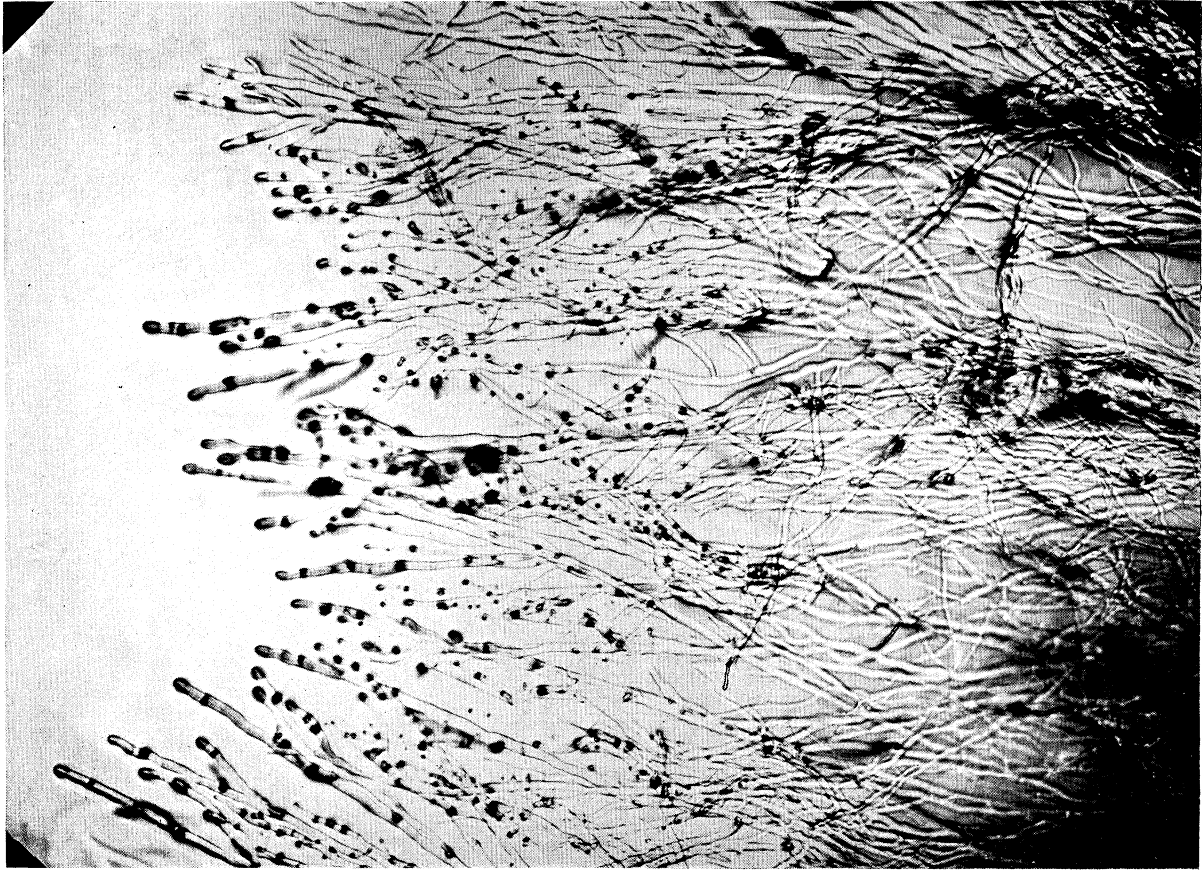

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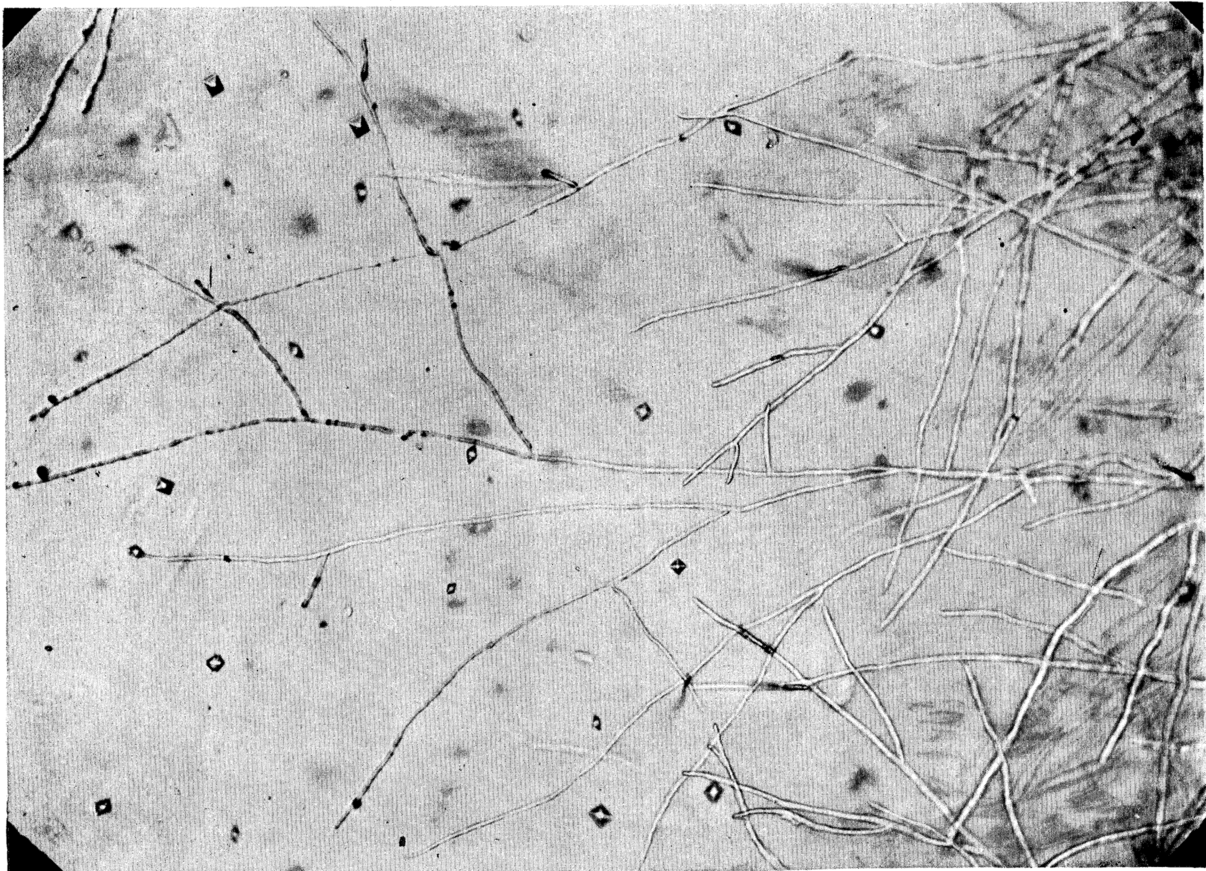

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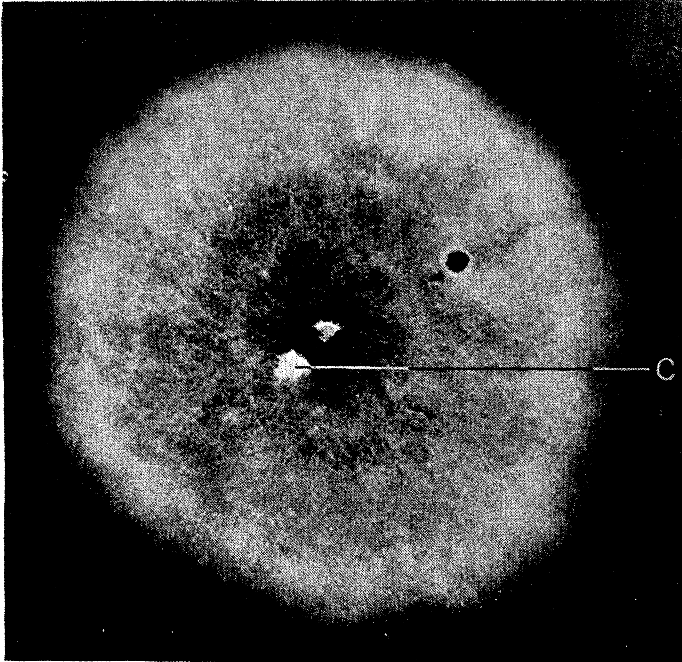

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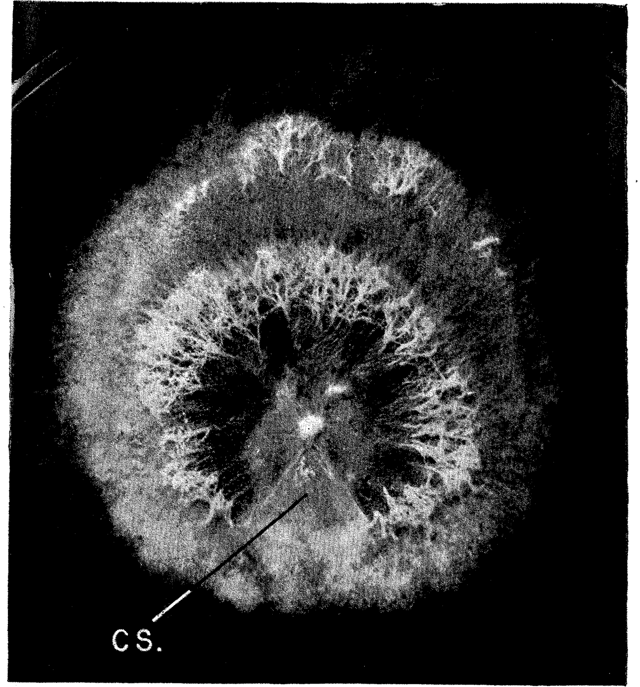

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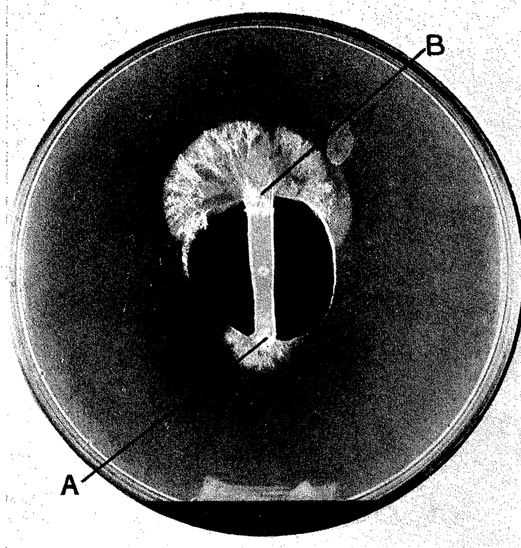


Fig. 21.

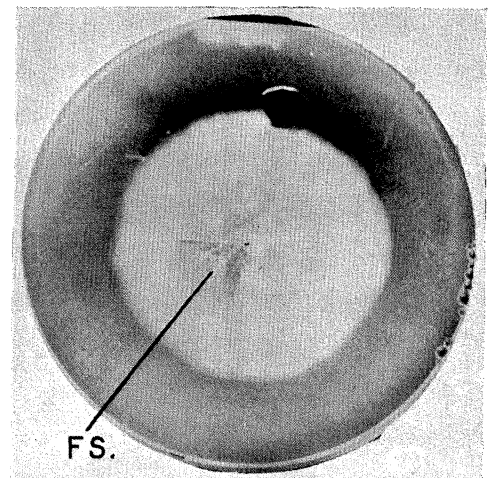
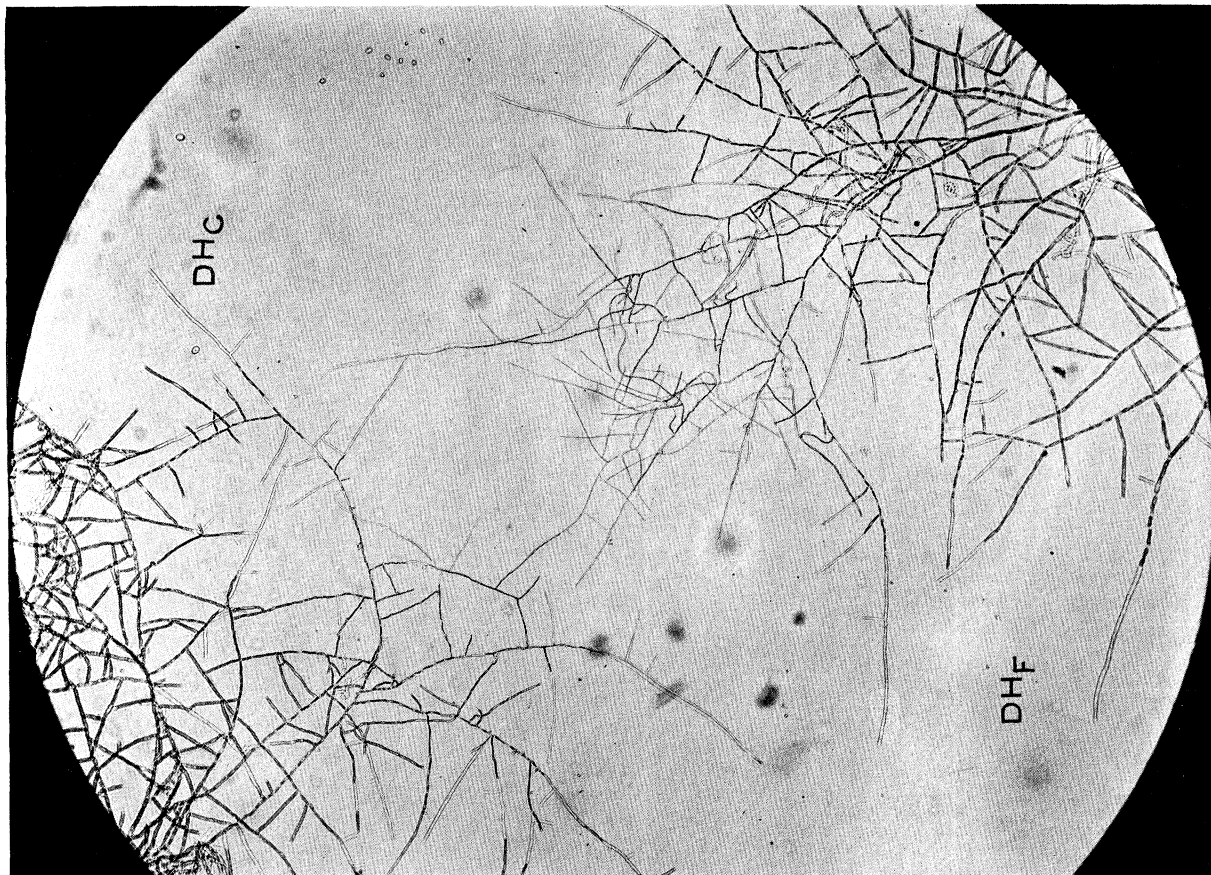


Fig. 22.



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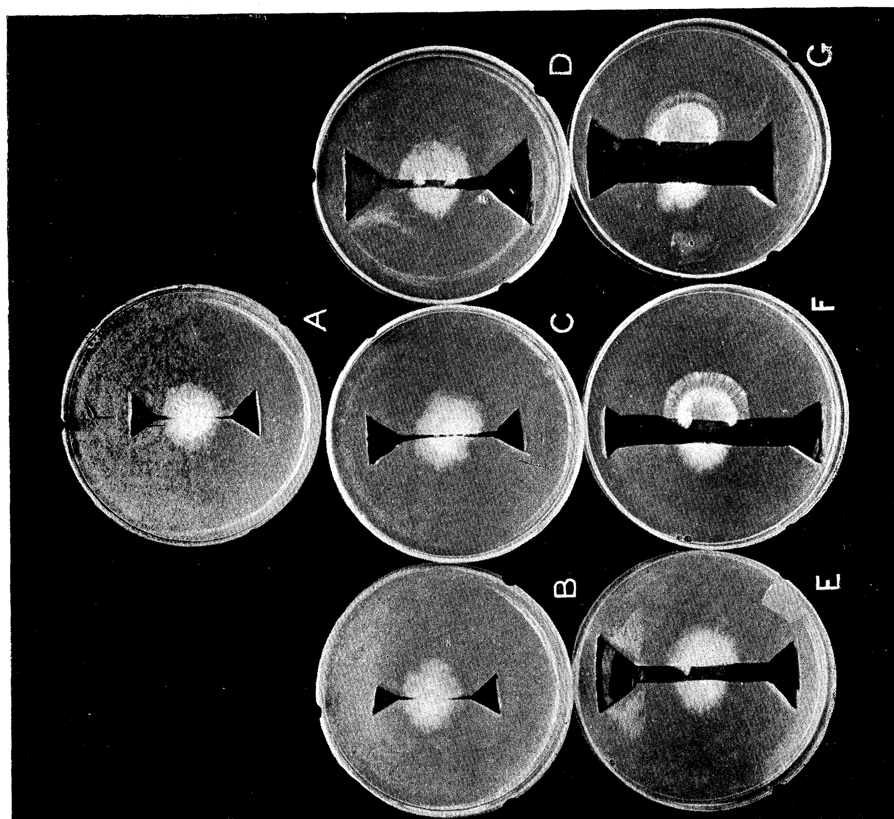
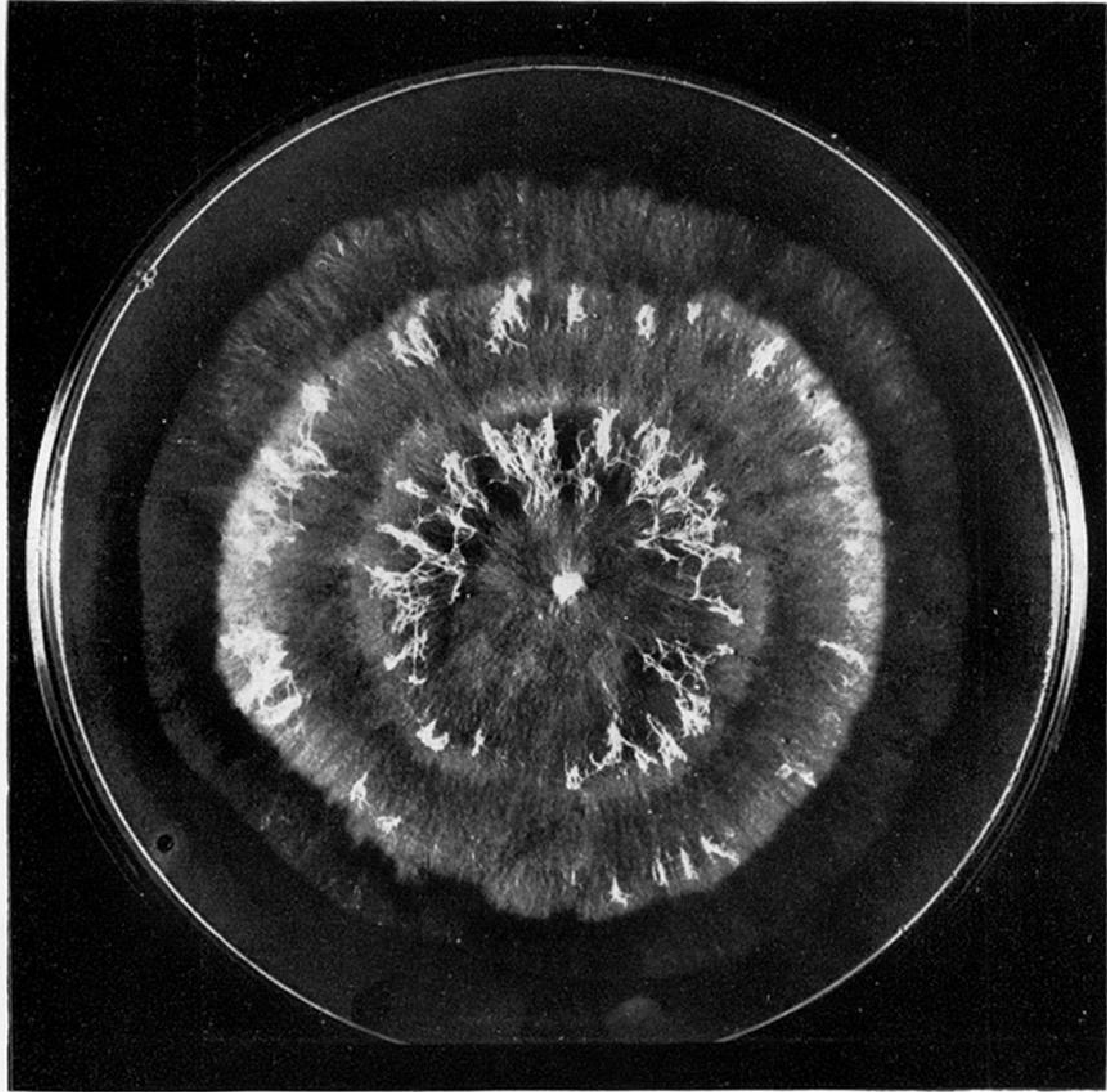


Fig. 23.

FIG. 22.—Growth character of a DH_C culture ten days after a sector had been removed and replaced by a similar sector of DH_F (F.S.). Radius at the time of the operation, 15·0 mm. Note that the growth from the sector is like DH_C. × $\frac{3}{5}$.

FIG. 23, A-G.—Illustration of the growth from inocula of DH_C and of DH_F, separated by a gap of width, (A) 0·5 mm., (B) 1·0 mm., (C) 2·0 mm., (D) 4·0 mm., (E) 6·0 mm., (F) 10·0 mm., (G) 15 mm. Note that the DH_C (left of gap) remained unchanged ; and that the DH_F (right of gap) was converted into DH_C in A, B, C, D, and E, but remained unchanged in F and G. × $\frac{1}{3}$.

FIG. 24.—Illustration of union between DH_C and DH_F hyphæ. Actual points of fusion cannot be recognized.



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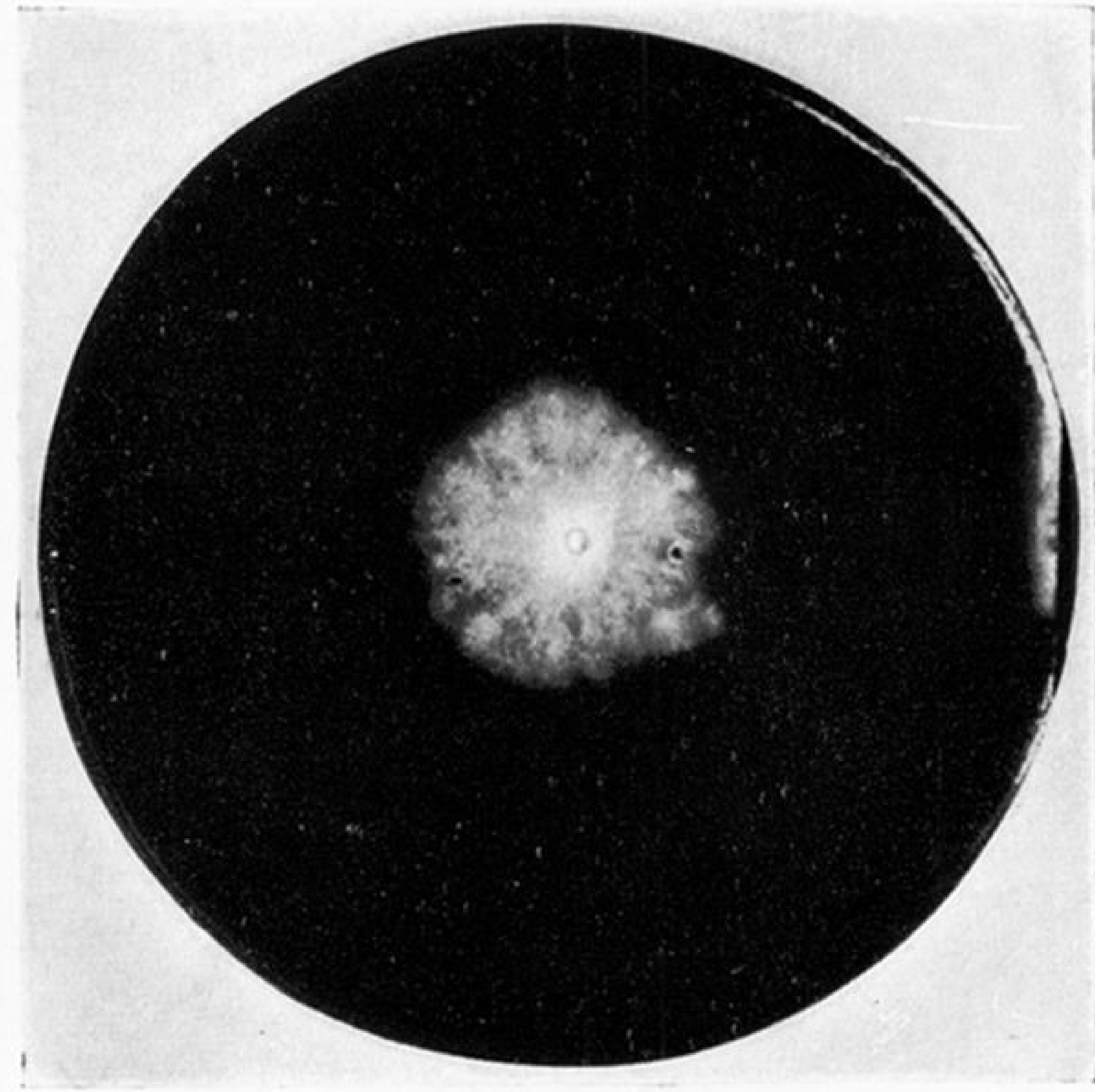

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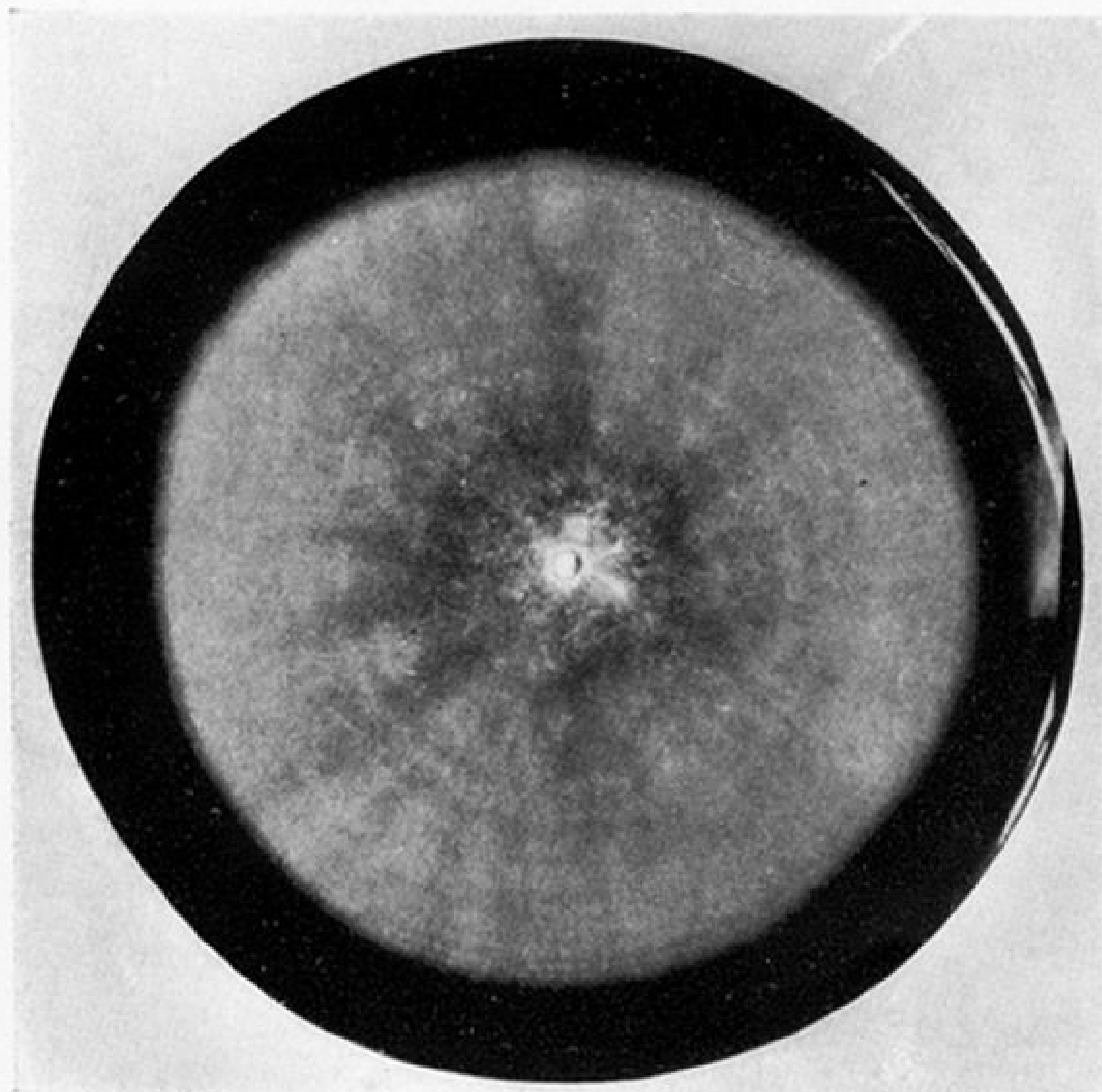

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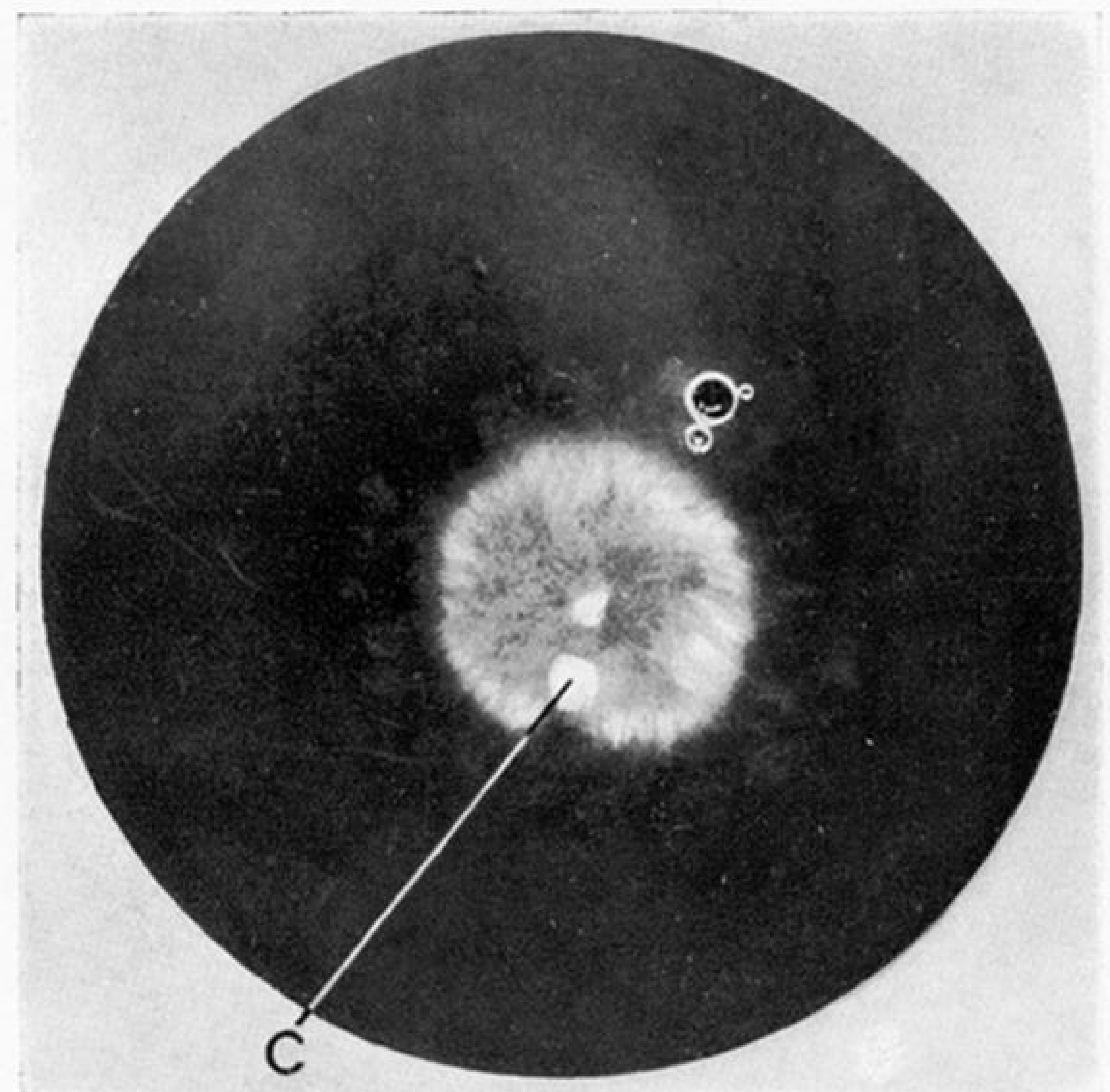

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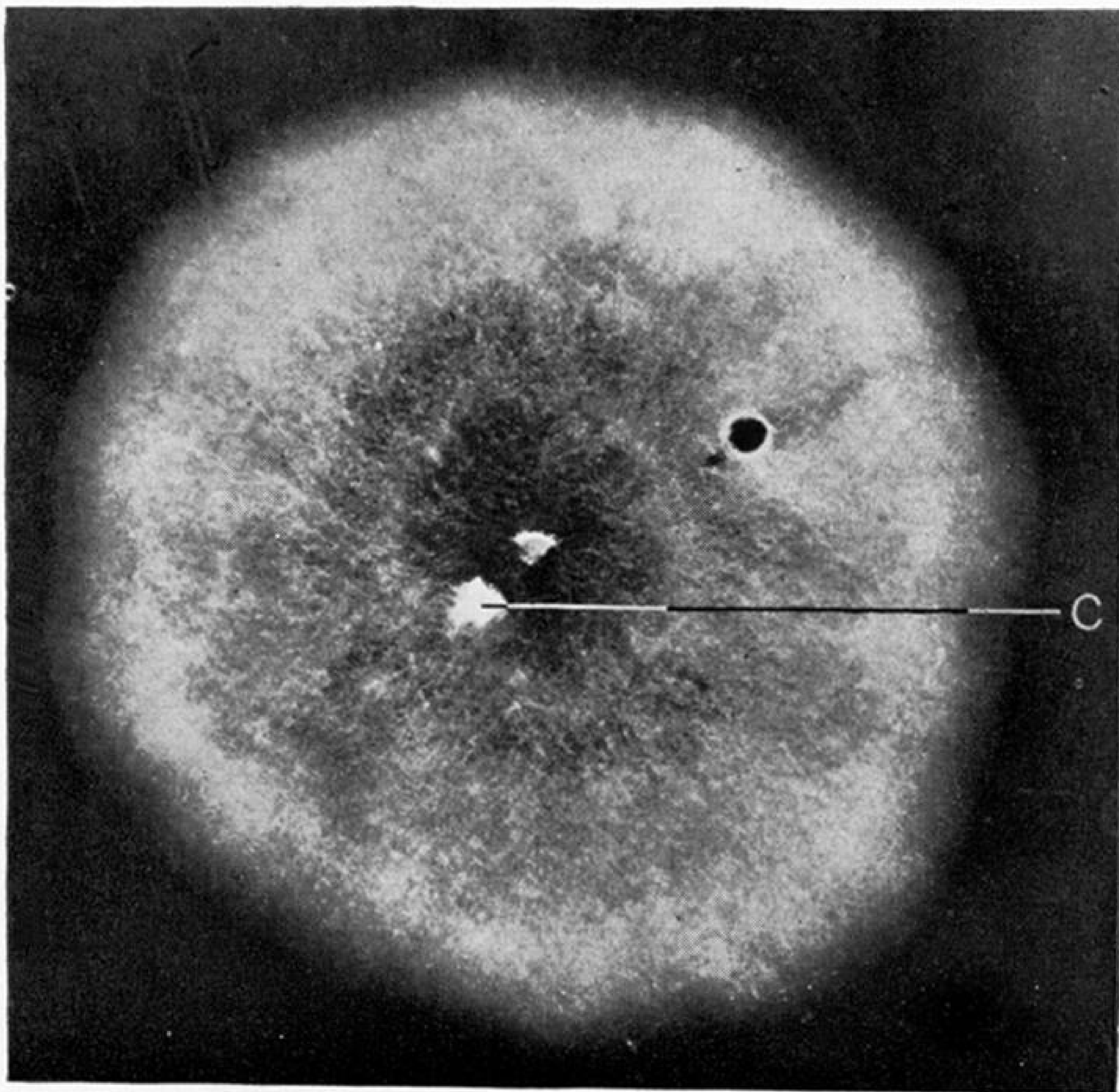

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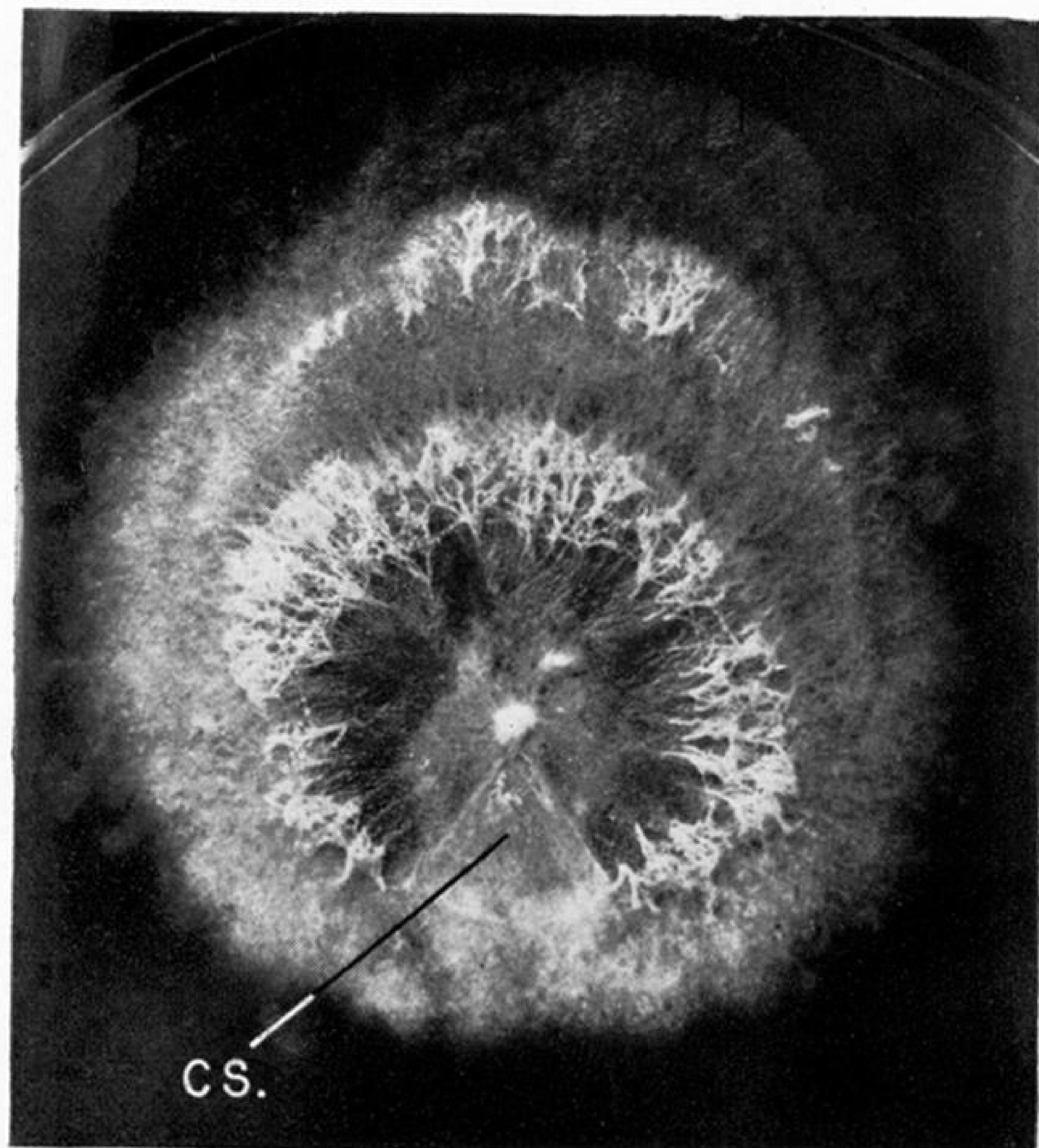

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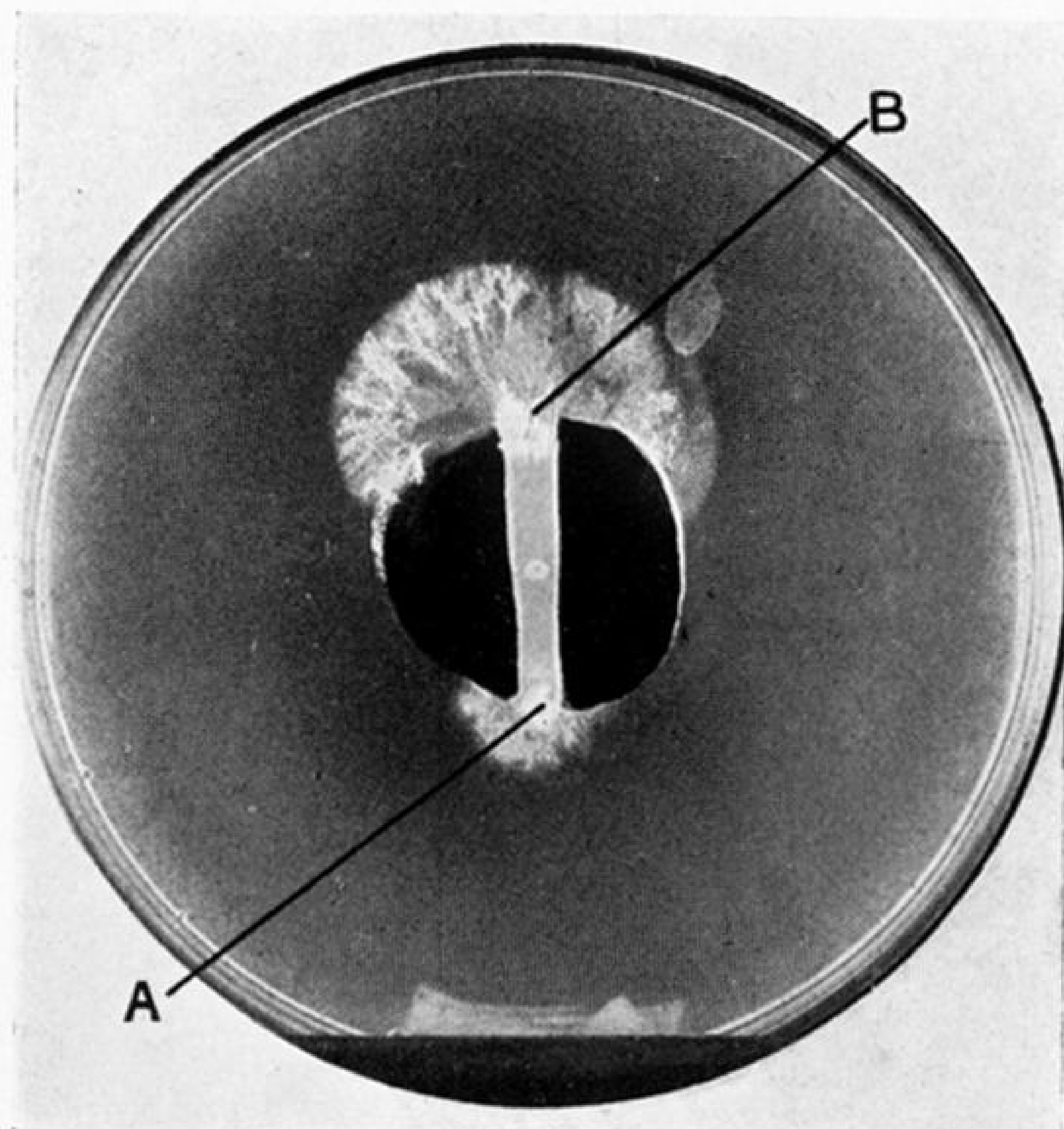

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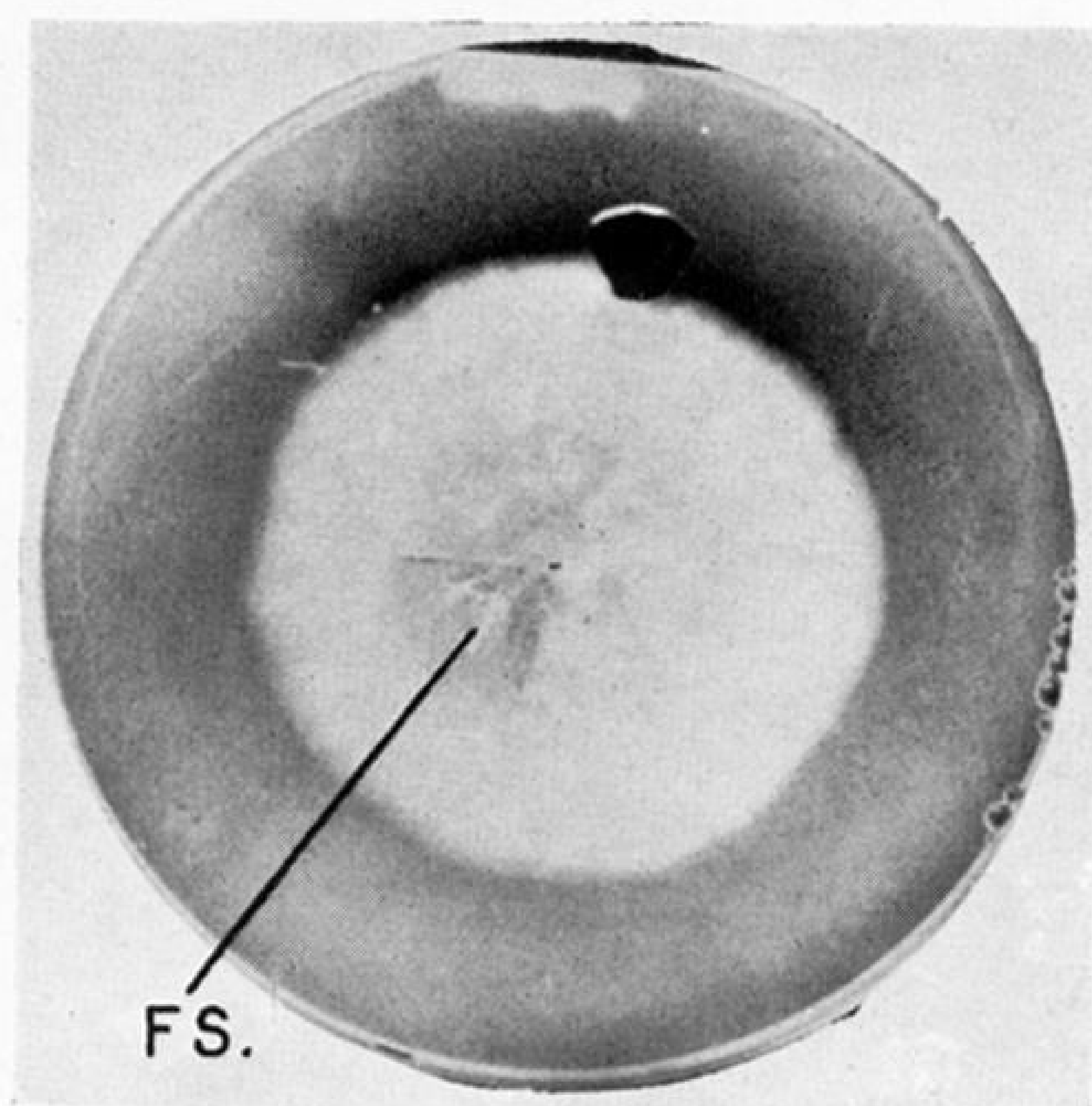

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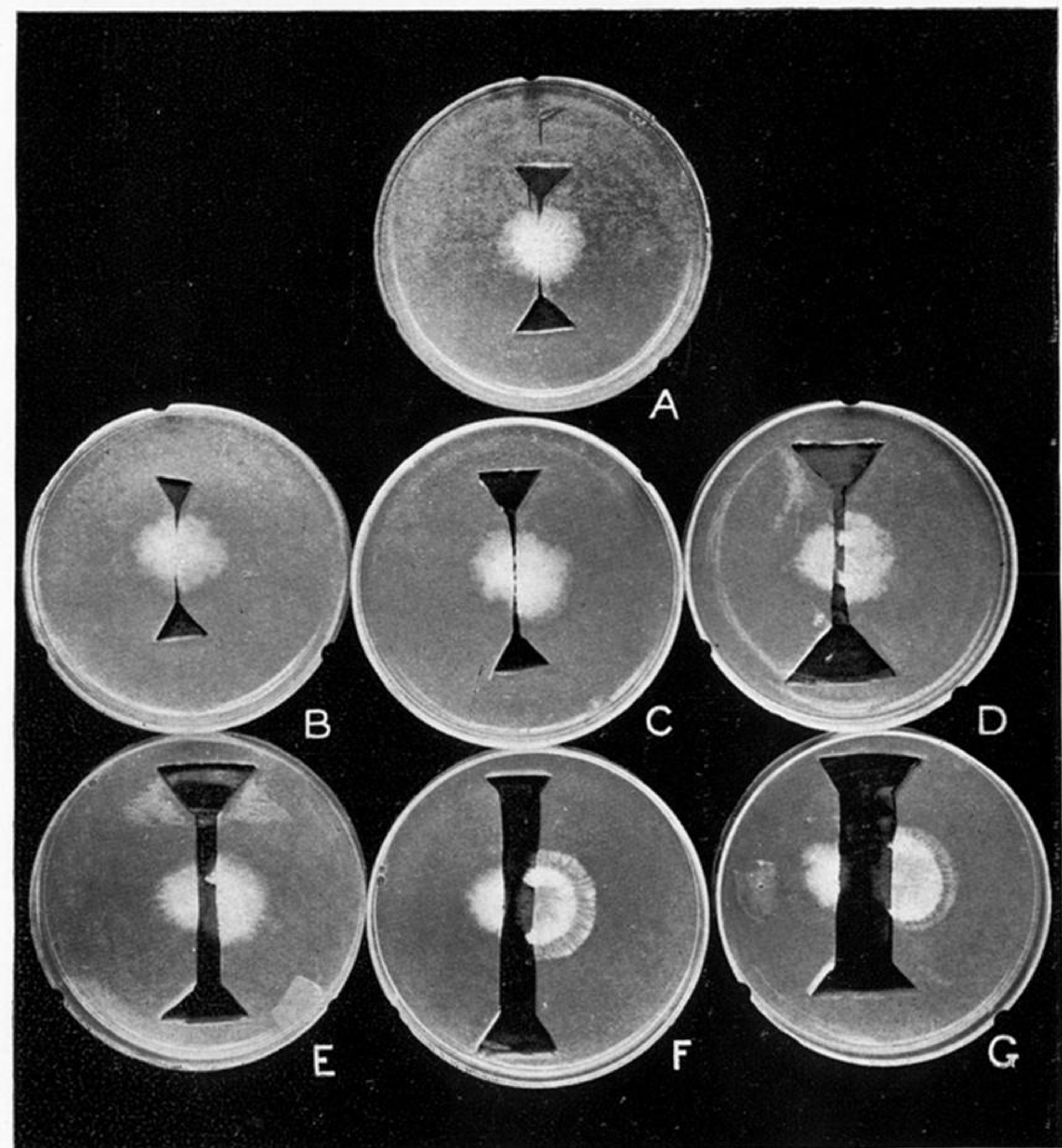

FIG. 23.

FIG. 24.