SUMMARY

In Part I the significance of the electrostatic charges on basidiospore is discussed. Measurements were made which confirmed the work of previous authors, who shewed that in the spore population derived from a single fruit body there tended to be an unequal number of positively and negatively charg spores. Estimation of the charge on the mean spore of populations of the dry rot fungus were made by allowing spores to fall through a horizontal electric field of known strength, measuring the horizontal displacement undergone by the spores, and then making subsequent estimations of spore mas For this a value of $(1.35 \pm 0.12) \times 10^{-8}$ e.s.u. was obtained. Charges of t order are likely to be acquired as a result of simple physical separation of the spore from the sterigma. It is also concluded that such charges are to small to have any significant influence on the liberation of spores from spec with narrow pores.

In Part II the influence of other forces on the liberation of spores fr narrow pores is considered. Fruit bodies were displaced from their normal orientation, and the decrease in spore liberation was recorded. It was fou that the decrease in liberation was that which was to be expected, if the spore trajectory were determined by the initial violent discharge and the gravitational attraction. Thus it is concluded that there were no extra-gravitational forces involved in spore liberation, other than the initial violent horizontal projection of the spore from the hymenium lining the pore wall.

Part III discusses previous work, which reported the presence of rhythmical spore patterns on the periphery of glass discs rotated slowly beneath fruit bodies of Trametes gibbosa. It is shown that these deposition patterns are determined by the operation of the heater of the incubator in which the experiments were carried out. However, other rhythmical shorter period (15 minute) spore deposition patterns have been found, which are not related to heater cycling or to other known variations in the environment. These patterns unrelated to heater cycling have not been consistently present. Some experiments to investigate these patterns have been carried out, and suggestions are made for further work.

SPORULATION IN FUNGI

WITH SPECIAL REFERENCE TO THE HYMENOMYCETES

A Thesis

presented for the Degree of Ph.D.

in the Faculty of Science in the University of Glasgow

by

James Taggart, B.Sc.

February
1961

"Benedicite omnia opera Domini Domino, hymnum dicite,

et superexaltate eum in saecula."

Daniel III, 57.

Contents

Contents (contd.)

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Introduction

The perpetuation and further evolution of a species demands the production of a new individual distinct from the parent in genetic constitution and in space. The former requirement is usually achieved by mutation and the sexual process, the latter by a variety of mechanisms depending on the nature of the units of dissemination. Attention in the present work is confined to the mechanism of dissemination of the hymenomycete basidiospore.

The dissemination of the basidiospore may be considered to take place in 4 successive stages.

(1) Liberation from the basidium.

(2) Liberation from the fruit body.

(3) Distribution in the atmosphere.

(4) Settling on the substratum.

Only the first two of these stages are considered in this thesis; these are examined particularly with regard to Polypores.

The remainder of this introduction gives a general outline of the process and indicates the topics which have been further investigated.

Nature and Numbers of the Units of Dissemination

The sizes of basidiospores have been extensively recorded for many years, being important taxonomic characters. The spores are characteristically oval with diameters of the order of 5 μ (for comparative sizes of spores see Ingold 1953). Measurement of, and variation in, spore size are discussed

briefly in Part I. An important attribute of the basidiospore is that it is of such a size to move in air in accordance with Stokes Law (Buller 1909, 1922), a point important in connexion with liberation from the fruit body.

Though the spores are very small, the numbers produced are vast (see discussion by Buller 1922). As this is characteristic of all species it seems that such spore production is necessary for the existance of these fungi. The numbers of spores produced by some species are considered in appendix A.

Discharge of Spores from the Basidium

The exogenously produced spores are attached by their apicula to the sterigmata. Buller (1909) demonstrated that they were violently discharged, but the mechanism by which this comes about is still uncertain.

Buller's observations shewed that the 4 spores are discharged successively at intervals of a few minutes or seconds. He observed a drop of liquid to be secreted at the junction of the apiculus and the sterigma a few seconds before discharge. This drop was carried away with the spore. Buller's initial hypothesis considered a cross wall to be present between the spore and the basidium and discharge to be the result of a rounding off process. However, being unable to convince himself of the presence of this wall he suggested that the propelling force was probably a localised hydrostatic explosion in a very narrow pore connecting the spore to the basidium. This hypothesis involves the breaking of the sterigma which does not, however, open out but remains turgid.

More recently two further theories have been amplified with regard to the discharge mechanism. Ingold (1939) shewed that the surface energy of

the drop of liquid is far higher than that required for spore discharge, and pointed out that this energy could "conceivably be used" in the process. He emphasised however that the suggestion was put forward only as a possibility, and that certain cases of abnormal spore discharge are "difficult to reconcile" with this view.

Prince (1943) working on Gymnosporangium nidus-avis detected a cross wall between the basidium and the spore. He considers a rounding off mechanism to be the explanation.

The newly discharged spores were shown by Buller (1909) to be electrically charged, and Gregory (1957) has investigated the distribution of sign of these charges in various species. No measurement had, however, been made of the magnitude of these charges. Part I describes experiments in which these charges are measured and, in the light of the results obtained, considers their possible effect on spore liberation from polypore fruit bodies.

Liberation of Spores from the Fruit Body

The relative ease with which spores can be liberated into the atmosphere will obviously depend on the complexity of the distribution of hymenium in the fruit body. In air spores may be expected to obey Stokes Law. Under gravity alone they may thus be expected to fall vertically at a rate which can be predicted from a knowledge of the size, mass and shape of the spore. Buller (1922) demonstrated this and considered that spore liberation from fruit bodies depends on the initial violent discharge and the gravitational attraction alone. However on such small particles within the confines of a complex fruit body there is the possibility of other forces contributing significantly to their movement (Ingold 1957). The electro-

static forces are considered in this context in Part I. Slight air currents, which can easily be demonstrated to have a considerable effect on spore movement (Buller 1909) may have some control over spore liberation (Falk 1904). The possibility of the existence of such non-gravitational forces to an appreciably extent with respect to the passage of spores down long narrow pores is considered in Part II.

Continuity of Spore Liberation

The period of spore liberation from a particular fruit body may be hours, days, months or years. The polyporaceae gives many examples of long lived and perennial fruit bodies (see e.g. Bjørnekaer 1937, Parmasto 1957). The reports on investigations of the uniformity of spore liberation rate are conflicting. For example, Buller (1922) concludes spore liberation rate to be uniform, whereas Barker (1910) reports an intermittent spore liberation. Part III gives an account of the investigation of this problem.

Part I. Electrostatic Charges on Basidiospores

Introduction

In physical systems it is known that small particles acquire electrical charges on separation from each other or their container (Loeb 1958). Where separation takes place between material of a single kind, the sign of the charge on any particle is determined by chance; but where materials of different kinds separate, there may be a tendency for the one material to acquire positive charges and the other negative. For example, when nickel powder is displaced from a quartz tube the nickel particles tend to become positively charged.

The charges on basidiospores were first noted by Buller (1909) who observed the fall of spores in a horizontal electric field (3,000 volts/cm.*) with a horizontal microscope. Some spores were displaced towards the positive and some towards the negative electrodes of the apparatus and a few spores fell vertically, apparently unaffected by the electric field. The rapidity of lateral displacement varied from spore to spore, but more moved to the one side than the other. The direction of movement of individual spores was reversed when the field was reversed. All spores were seen to fall vertically when no field was applied. These observations were made on Psalliota campestris Fr. (Agaricus campestris), Polyporus squamosus (Huds.) Fr. and other species.

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* This value is derived from information obtained from the Electricity Board regarding the power supply to Birmingham University at the time when Buller carried out his experiments.

Table I

Signs of Charges on Basidiospores recorded by Previous Authors

Species	Predominant Sign	Authority
Agaricus campestris	+ ve	Gregory 1957
" "	- ve	Buller 1909
Coprinus micaceus	- ve	Gregory 1957
Coprinus hiascens	- ve	"
Polyporus squamosus	- ve	"
Flammula carbonaria	+ ve	"
Pholiota squarrosa	+ ve	"
Ganoderma applanatum	+ ve	"

Gregory (1957) using essentially similar apparatus, made observations in the field on the charges on the spores of Ganoderma applanatum (Pers.) Pat. and other species (see Table I). Observations in these experiments were made on the density of deposit adhering to the charged plates maintaining the electric field. The results again suggested that each fruit body and each species produced spores bearing charges predominantly of one sign. A field of 200 volts/cm. was used.

Asymmetrical distribution of charge has also been demonstrated in the Ascomycetes for Lophodermium pinastri Schnad. Chev. (Rack 1959). Spores in the atmosphere appear to be positively charged but some evidence is given indicative of predominantly negative charges immediately on discharge.

The previous investigations have demonstrated the asymmetrical distribution of charges in spore populations but not the order of magnitude of the charges. It is, however, necessary to assess the order of magnitude if the effect of the charges on spore movement in confined spaces (such as within pores) is to be considered. In this context the important quantity affecting the movement of free spores is their ratio of charge to mass. This ratio is determined experimentally and separate estimates of spore mass enable the absolute charge on a spore to be calculated.

The method is based on the theoretical considerations below.

Theoretical Considerations

The charge / mass ratio of a particle may be determined from its path through a viscous medium (air) under the action of crossed, known gravitational and electrostatic fields (Hopper and Laby 1941, see also Loeb l.c.).

For a spherical particle moving through a viscous medium with uniform velocity Stoke's Law gives:

$F = 6 \pi \eta a v$ where F = the force acting on the particle.

i.e. $v = \frac{F}{6\pi\eta a v}$ η = the coefficient of viscosity.

a = the radius of the particle.

v = the velocity of the particle.

Consider a spherical particle falling under gravity in a horizontal electri field.

The vertical force acting on the particle will be m x g

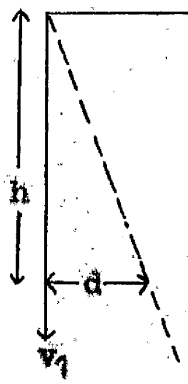
where m = the mass of the particle.

g = the acceleration due to gravity.

The horizontal force acting on the particle will be e x E

where e = the charge on the spore.

E = the field strength.



Let vertical component velocity be v_1

" horizontal " " " " v_2

Then applying Stoke's Law.

$v_1 = \frac{m g}{6\pi\eta a}$ and $v_2 = \frac{e E}{6\pi\eta a}$

$\therefore \frac{v_2}{v_1} = \frac{e E}{m g}$

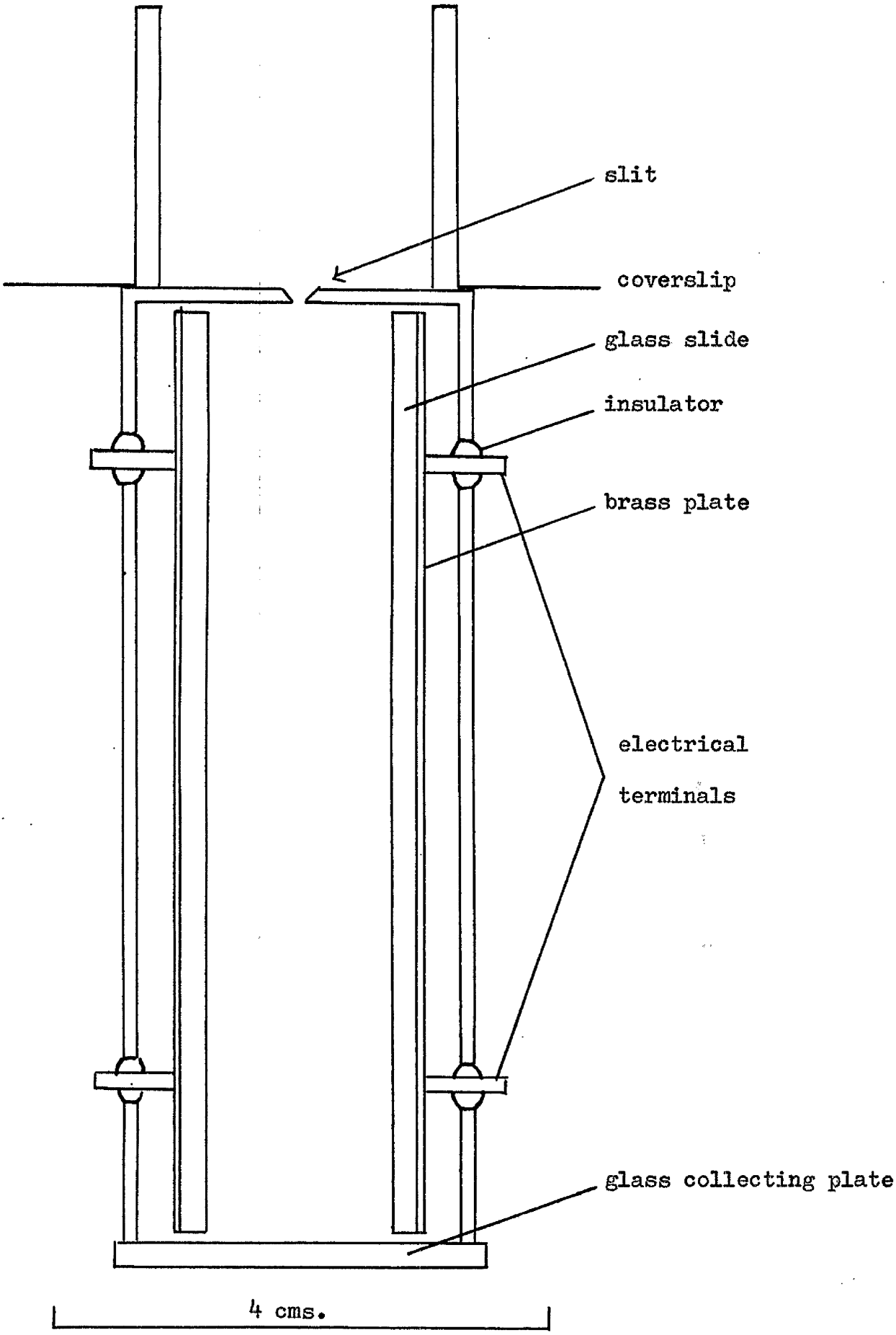
Now if the vertical distance of particle fall be h, the horizontal displacement be d and the time taken to attain these displacements be t,

then $\frac{v_2}{v_1} = \frac{d}{t} \times \frac{t}{h} = \frac{d}{h} = \frac{E e}{m g}$

i.e. $\frac{e}{m} = \frac{d g}{E h}$

Figure 1.

APPARATUS FOR DISPLACEMENT OF SPORE IN AN ELECTRIC FIELD



If the particle is not quite spherical it may be expected that a similar expression will apply for Stoke's Law with some modification of the numerical constant and with the radius replaced by some other typical dimension. It may also be noted that neither the numerical constant nor the value for the radius appears in the final expression for $\frac{e}{m}$. It is thus reasonable to suppose that the results will not be affected by the ellipsoidal form of the spores.

Thus it is possible to determine the $\frac{e}{m}$ ratio of a spore by letting it fall through a known distance (h) and measuring its horizontal displacement (d) under the influence of a known uniform electric field F.

The charge on the spore (e) can then be calculated on determination of the mass.

Material

The dry rot fungus (Serpula lacrimans Pers. ex S.F. Gray) was used by reason of its abundance and availability throughout the year. As it usually proved impossible to obtain fruit bodies adhering to their substrates, they were supported when used in the experimental work on galvanised wire shelves. The spores of this species are oval, measuring about 8 x 5μ.

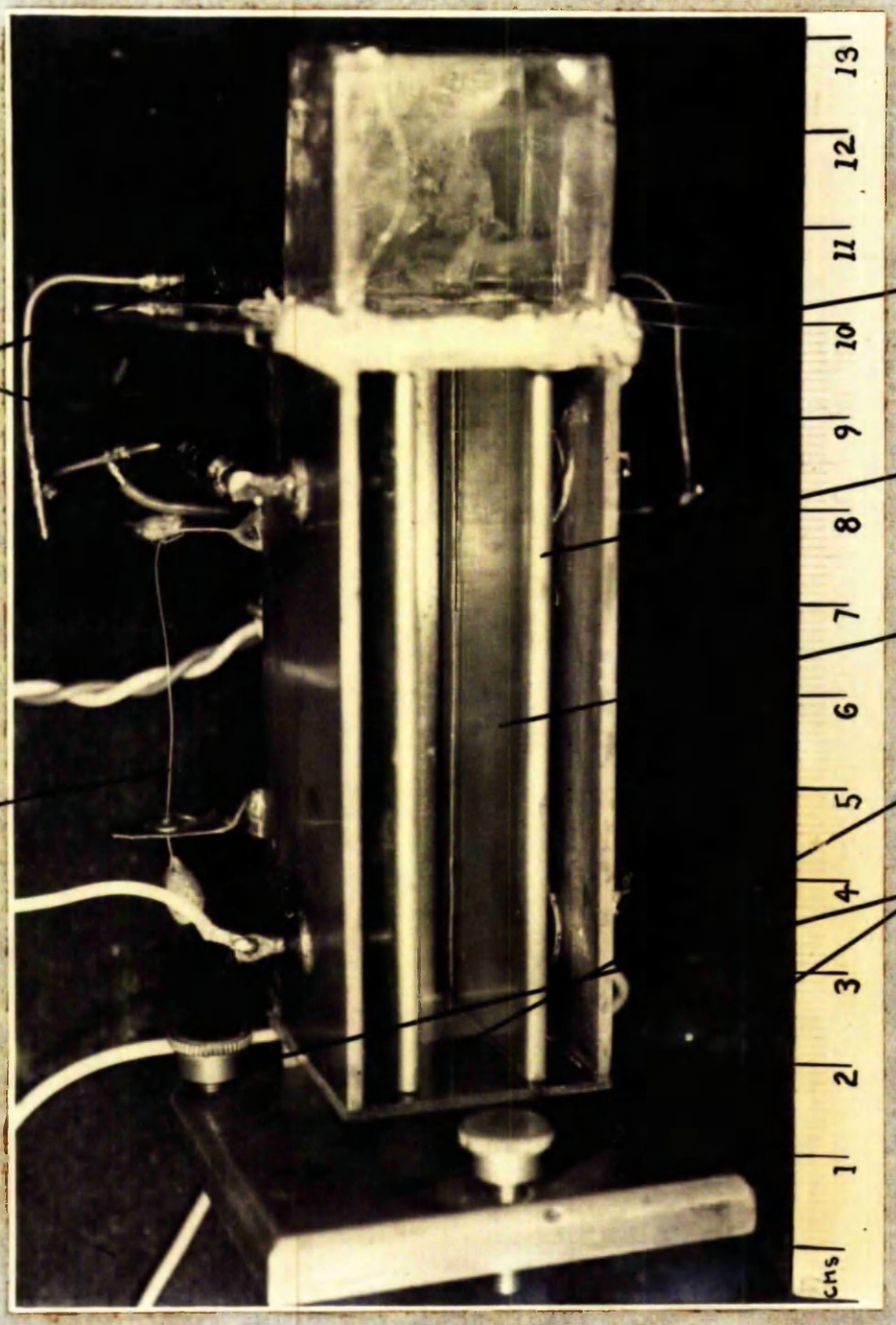
Determination of Charge/Mass Ratio

(a) Apparatus and Method

The apparatus is shewn in figure 1. It is a modification of that used by Gregory (l.c.) suggested by the experiments of Hopper and Laby (l.c.). It consists of a brass box 77 = 26 x 32 mm. in which vertical brass plates, 75 x 26 mm. are supported on insulators on opposite sides, their inner

Photograph 1.

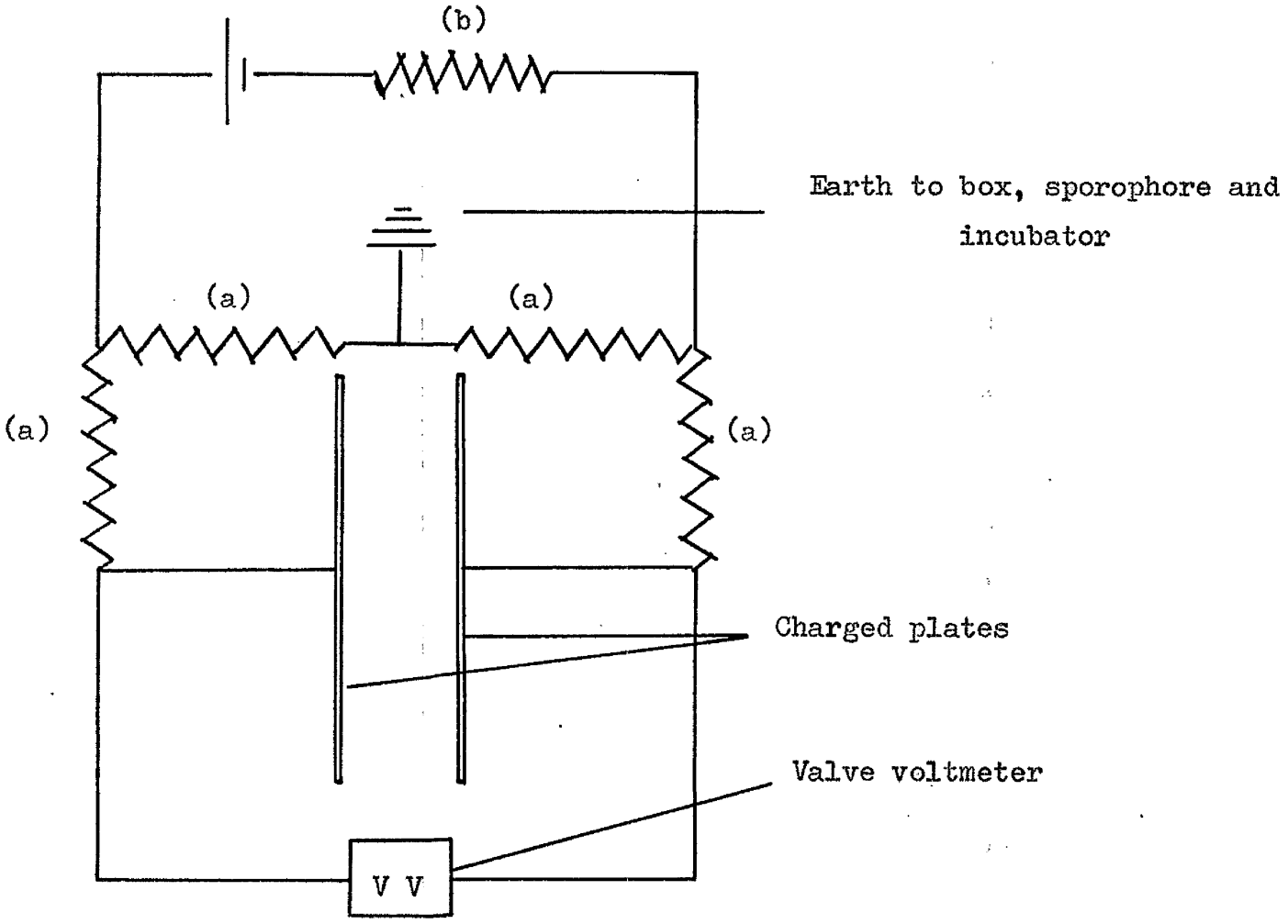
Apparatus for displacement of spores in an electric field



* The bend in the plumb line results from the apparatus being photographed on its back.

NOTE. The front cover has been removed.

Figure 2. ELECTRICAL CONNEXIONS



Resistors (a) of $1.2 \times 10^6 \Omega$ to maintain symmetry about earth

surfaces being 7.5 mm. from the box centre. Their inner sides are covered by glass slides. Spores enter the box through a slit, 12 x 0.75 mm., parallel to, and midway between, the plates. The bottom of the box is covered with a slip of glass on which the spores collect.

The fruit body is supported about 25 mm. above the slit, to allow the spores to reach their steady terminal velocity before passing through it (cf. Buller 1909). The intervening space is enclosed by glass plates to prevent draughts. Coverslips are fixed projecting over the plate terminals to protect them from moisture dripping from the fruit body. The whole assembly is mounted on levelling screws and a plumb line is attached to its side. (Photograph 1).

Figure 2 shews the electrical connexions. The $1.2 \times 10^6 \Omega$ resistors (marked (a) in the diagram) serve to maintain electrical symmetry of the plates about earth. The resistor (b) is such that a satisfactory order of displacement of the spores is obtained using three 45 volt radio batteries connected in series. During the experiment a valve voltmeter is connected to the plates as monitor.

The fungus and apparatus are placed in, and earthed to, a copper lined waterjacketed incubator held at 20°C.

The atmosphere inside the incubator is kept as nearly saturated as possible. The apparatus is levelled and the spores allowed to fall for a day or more.

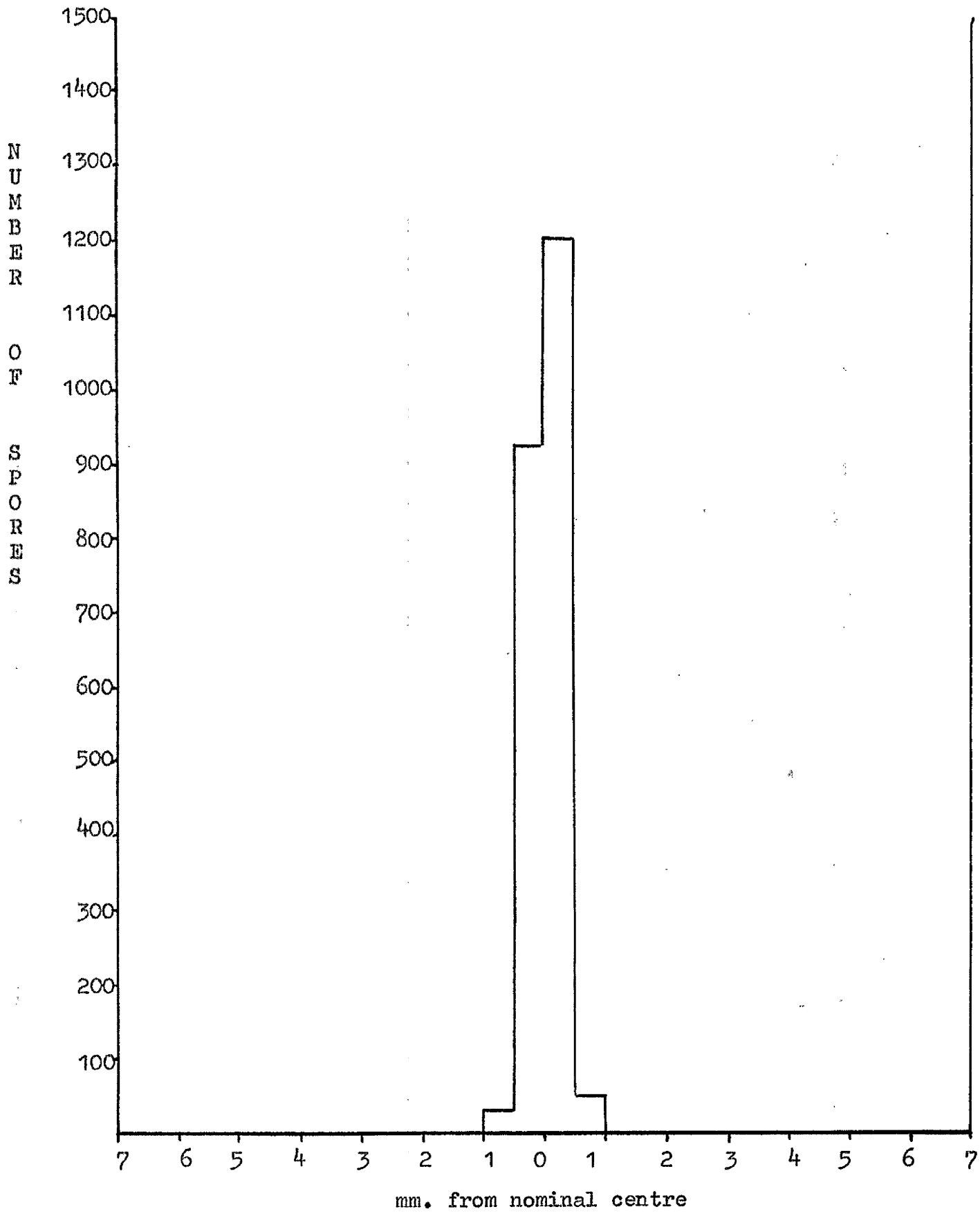
At the end of the experiment the distribution of spores on the base plate of the apparatus is determined by counting the spores per unit area at ½ or ¼ mm. intervals. The unit area chosen for counting depends on the overall density of spores in particular cases. In critical cases some

Photograph 2.

Deposit on Base Plate - No Field Applied

x 4

Figure 3. No Field Applied



40,000 spores are counted to obtain distribution curves.

Experiments were carried out under the following conditions:

(1) No field applied.

(2) Field applied, in one direction throughout the experiment.

(3) Field applied, direction reversed at regular intervals (every few hours) throughout the experiment.

The first type of experiment gives an indication of the degree to which spores fall vertically when no lateral field is applied. The second type gives information as to the predominant sign of the charges, and a measure of the width of the distribution of displacement in the spore population. This type would also enable the mean displacement to be determined if the position of the vertical projection of the slit on the base plate were known. The difficulty of determining the position of the slit projection is overcome in the experiment in which the field is reversed. Here two superimposed mirror image distributions are to be expected, and thus by measuring the distance between the mean displacement for each half distribution a value for 2d can be obtained without a knowledge of the projection of the slit.

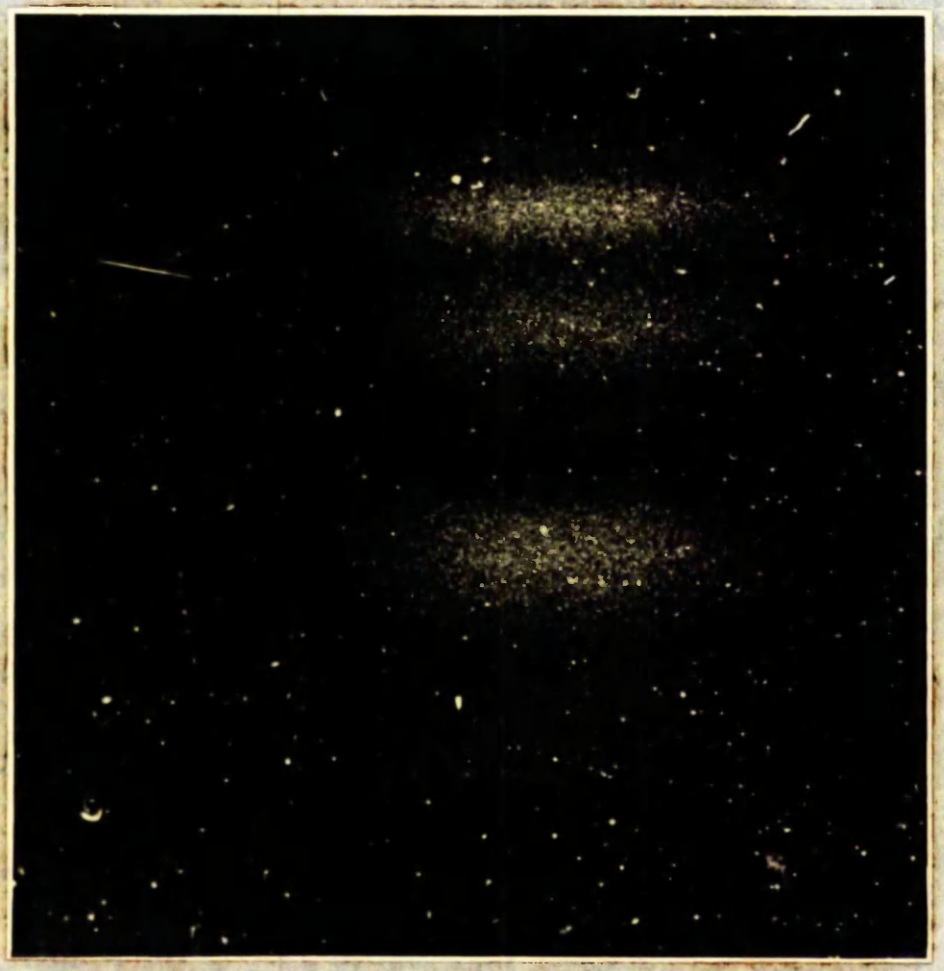
(b) Results

(1) Experiments with no Field Applied

Photograph 2 shews the deposit on the base plate still attached to the apparatus at the end of such an experiment. The outline of the slit is seen out of focus behind the spore deposit. The distribution at right angles to the slit is shewn graphically in figure 3. It is concluded from such experiments that spore fall is approximately vertical.

Photograph 3.

Deposit on Base Plate - Field Applied.



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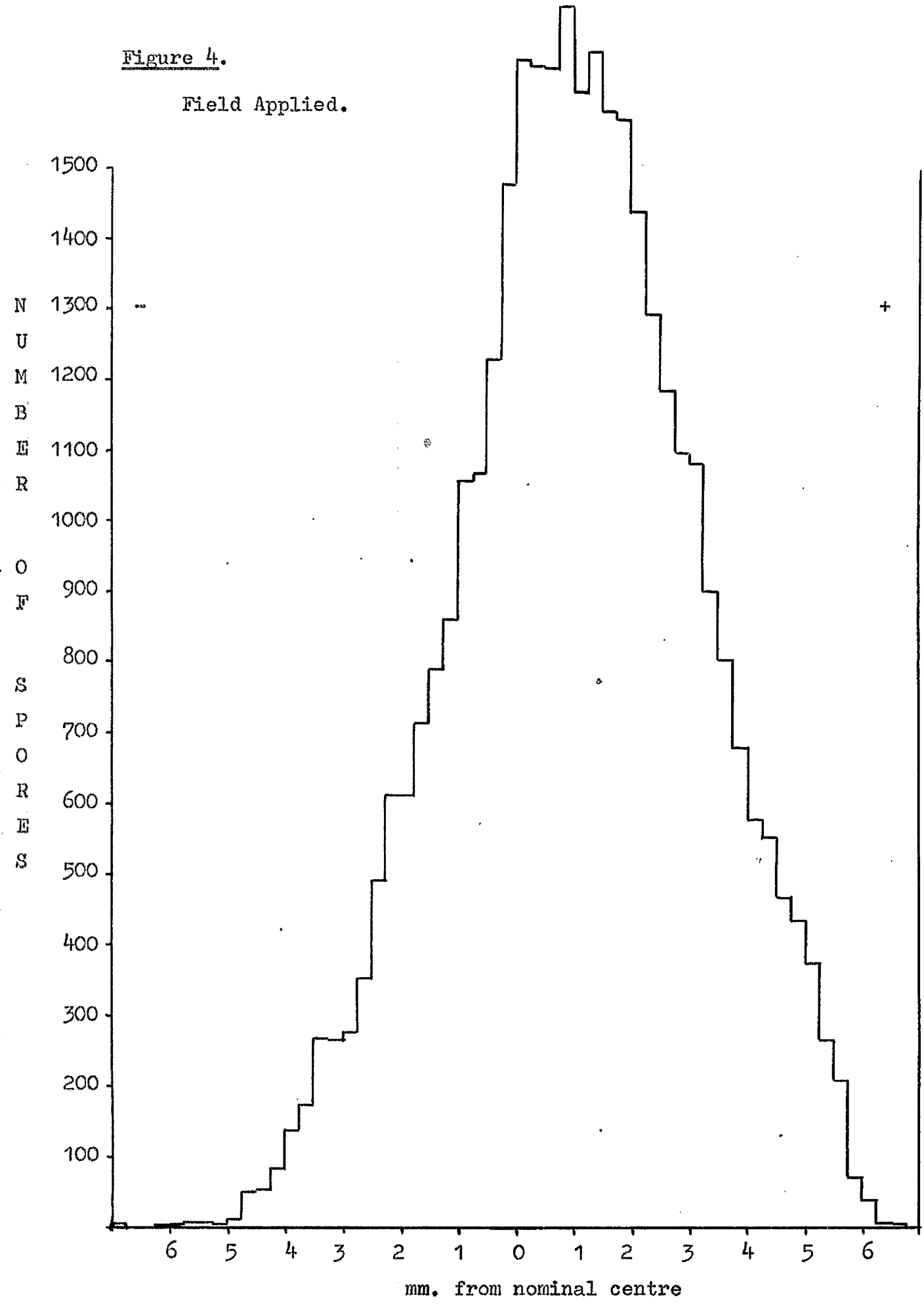
\+

x 5

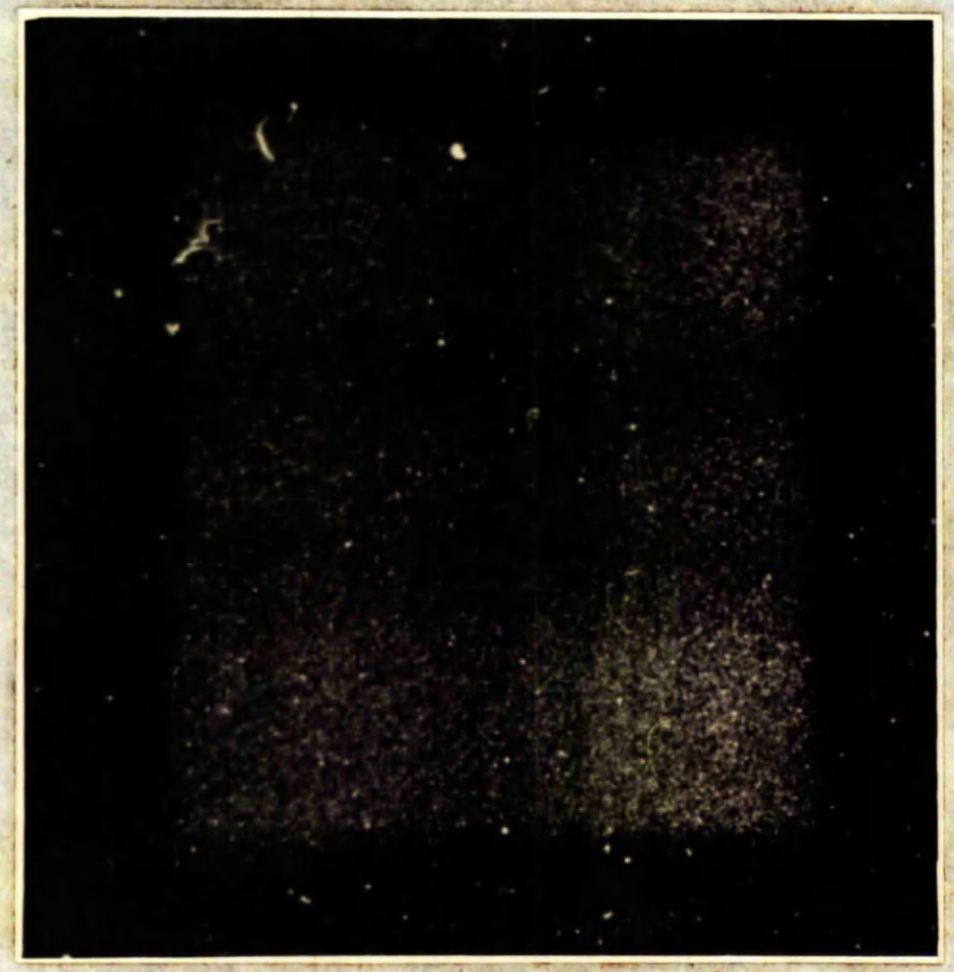
Figure 4.

Field Applied.



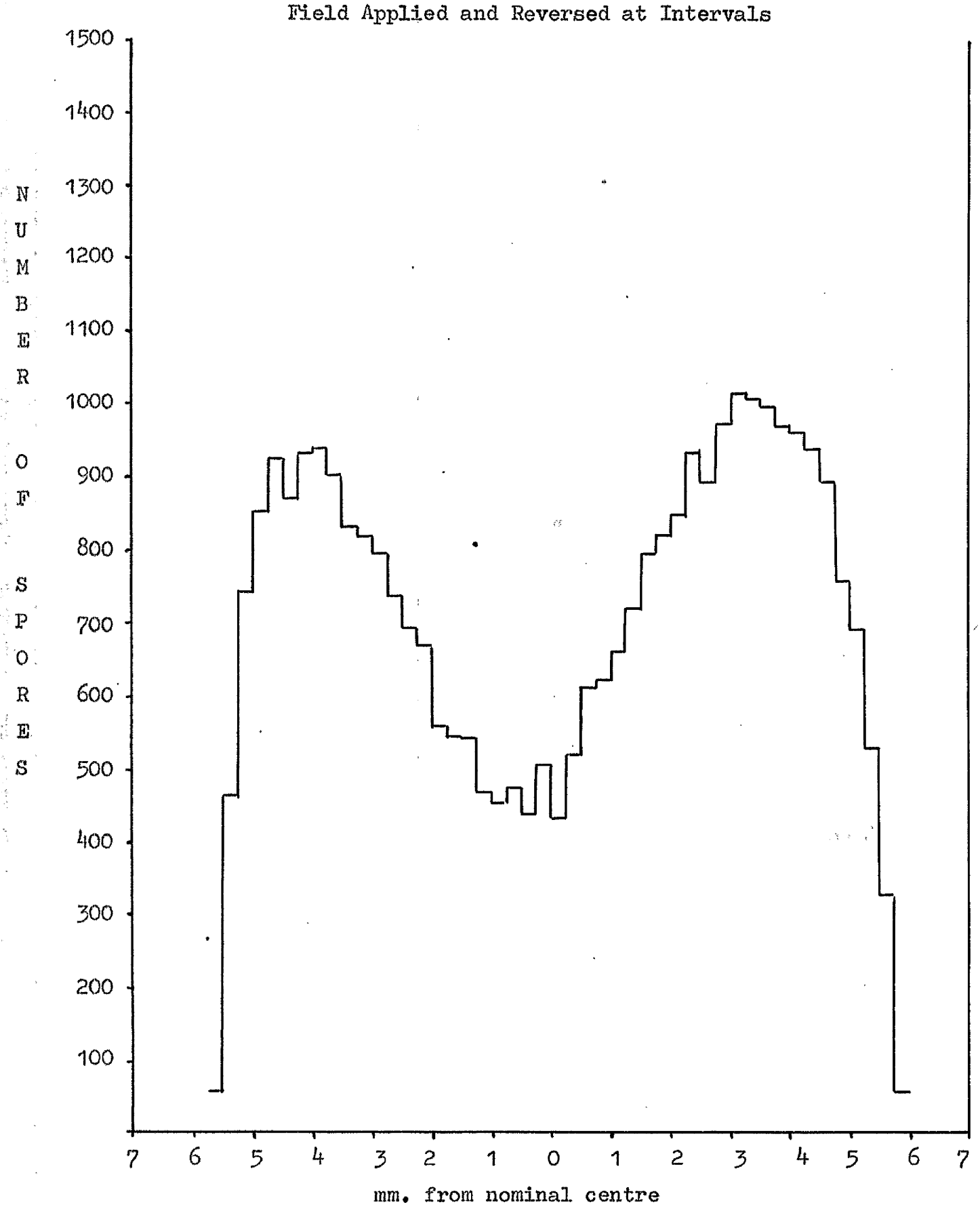
Photograph 4.

Deposit on Base Plate - Field Applied and Reversed at Intervals



x 5

Figure 5.


Field Applied and Reversed at Intervals
NUMBER OF SPORES
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
7 6 5 4 3 2 1 0 1 2 3 4 5 6 7
mm. from nominal centre

(2) Experiments with Field Applied but not Reversed

The deposit at the end of such an experiment is shewn in photograph 3 and the distribution at right angles to the slit graphically in figure 4. Here the spores shew a wide dispersal over the base plate, but the densest part of the deposit is nearer the positive side, thus indicating that a majority of these spores carry a negative charge (see appendix B). The standard deviation of this distribution is 2.13 mm. It is noted that this distribution is slightly skew. Skewness, defined as the square of the third moment divided by the cube of the second moment (see e.g. Weatherburn 1949) = 3.4×10^{-3} mm.

This distribution agrees well with those found in other experiments.

(3) Experiment with Field Applied but Reversed at Intervals

The deposit at the end of the experiment is shewn in photograph 4 and the distribution at right angles to the slit graphically in figure 5. Two dense regions can be seen on either side of the slit position. The valve voltmeter shewed a steady potential throughout this experiment of 84 volts. This experiment was followed by one with no field applied which gave a result similar to that shewn in photograph 2.

(c) Calculation of Charge / Mass Ratio

Since the images of the slit, shewn by the deposits on the base plates in experiments in which no field was applied, indicate that the lateral displacement in the other experiments with a field applied is due to the field alone, we can interpret the distributions across the base plates in terms of the charge/mass ratio (see above).

On the basis of the measurements obtained from the experiments described above the calculation proceeds as below.

$$\frac{e}{m} = \frac{dg}{hE} \qquad d = \left(\frac{0.73}{2} \pm 0.213^{*}\right) \text{ cms.}$$

$$g = 981 \text{ cms/sec}^2$$

$$h = 7.85 \text{ cms.}$$

$$E = \frac{84}{1.5 \times 300} \text{ e.s.u./cm.}$$

$$\frac{e}{m} = \frac{\left(\frac{0.73}{2} \pm 0.213\right) \times 981 \times 1.5 \times 300}{7.85 \times 84}$$

$$= 245 \pm 143 \text{ e.s.u./gm.}$$

* This value for the standard deviation is that of the distribution illustrated above as representative of experiments in which the charges were not reversed. The value is close to that obtained in other experiments of the same kind.

Determination of Spore Mass

Three methods were considered for this work: (a) the estimation of the numbers of spores in weighed samples, (b) measurement of volume, and (c) interference microscopy.

Method (a), Direct Weighing

The spores used in these estimations were collected on glass plates. When first collected the deposits were visibly damp, the fruit bodies having guttated moisture. The deposits were therefore left for some hours to equilibrate with the room atmosphere before being put into corked specimen tubes to await weighing.

Table II

Source	'e'/a fruit body				Other fruit bodies							
No. Sample	1		2		3		4		5		6	
Wt. of Sample	0.03240 g.		0.02715 g.		0.02470 g.		0.02120 g.		0.02270 g.		0.01510 g.	
	a	b	a	b	a	b	a	b	a	b	a	b
1	263	4.93	229	4.74	184	5.37	131	6.47	137	6.63	103	5.86
2	215	6.03	171	6.35	148	6.68	135	6.28	132	6.88	85	7.11
3	228	5.68	197	5.51	182	5.43	156	5.44	126	7.21	94	6.43
Count 4	206	6.29	162	6.70	139	7.11	163	5.20	172	5.28	118	5.12
5	223	5.81	245	4.43	176	5.61	136	6.24	151	6.01	121	4.99
6	236	5.49	195	5.57	133	7.43	128	6.63	182	4.99	101	5.98
Number 7	193	6.72	249	4.36	180	5.49	140	6.06	121	7.50	111	5.44
8	224	5.79	204	5.32	181	5.46	107	7.93	129	7.04	97	6.23
9	245	5.29	250	4.34	173	5.71	134	6.33	183	4.42	80	7.55
10	215	6.03	218	4.98	164	6.02	121	7.01	163	5.57	106	5.70
Mean Spore wt.	5.81×10^{-11} g.		5.23×10^{-11} g.		6.03×10^{-11} g.		6.36×10^{-11} g.		6.15×10^{-11} g.		6.04×10^{-11} g.	

a - Number of Spores.

b - Spore Mass x 10^{-11} g.

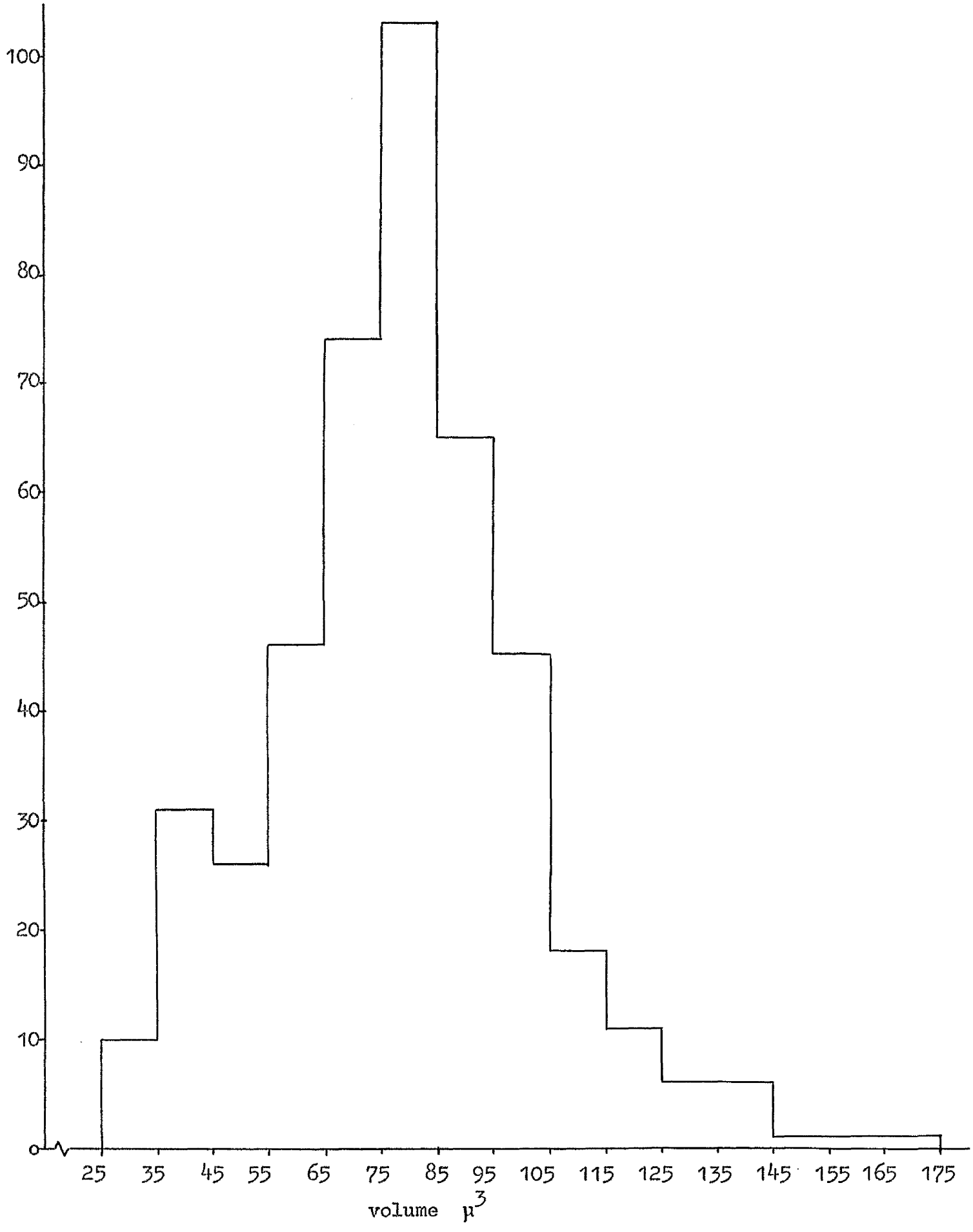
Samples of about 0.02 gms. were weighed to the nearest 0.00005 gms. in 20 cc. specimen tubes in equilibrium with the room atmosphere. All weighings were carried out at least twice. In each case the weighing of the tube + spores followed immediately the weighing of the empty tube.

The weighed samples were suspended in 1 cc. of methylated spirit, an even suspension being obtained by repeatedly passing the suspension through a fine pipette. The suspension was then made up to 10 cc. and shaken vigorously in a wrist action shaker. Ten haemocytometer counts were made for each sample, the sixteen small squares at the intersections of the large squares of the Thoma grid being counted in each instance.

Table II shews the results for spores obtained from the fruit body used in the $\frac{e}{m}$' experiment and from a mixed spore population from other fruit bodies obtained from the same house. Analysis shews that the variance ratio between these two lots of spores is too great for them reasonably to be regarded as belonging to a single population ($F = 2.95$). The mean value of the two estimates for the $\frac{e}{m}$' spores is therefore used in the calculation of e from the $\frac{e}{m}$ determination. This value is 5.52×10^{-11} gms. with 95% fiducial limits, based on the within column variance for all six estimations, of $\pm 0.481 \times 10^{-11}$ gms. Other results based on mixed populations from several fruit bodies gave mean values of 7.89×10^{-11} gms. (95% fiducial limits $\pm 0.585 \times 10^{-11}$ gms.) and 6.59×10^{-11} gms. (95% fiducial limits $\pm 0.704 \times 10^{-11}$ gms.).

It is noted that this method gives no estimate of variation in spore mass within a population, but only an estimate of the error in determining the mass of the mean spore.

Figure 6.


NUMBER OF SPORES
100
90
80
70
60
50
40
30
20
10
0
25
35
45
55
65
75
85
95
105
115
125
135
145
155
165
175
volume μ^3

Method (b). Measurement of Volume

Measurements were made of 442 dry spores on glass with a moving hair eyepiece micrometer. These spores were obtained from a single fruit body. A magnification of about x500 was used.

The frequency of the various volume classes derived from these measurements is shewn in figure 6. The mean spore volume is 78.1 μ^3 ($\sigma = \pm 22.5\ \mu^3$). The standard deviation expected from error in measurement has been estimated by repeated measurements of individual spores and is of the order of $\pm 14\ \mu^3$. It is concluded that this error in measurement is such that the results cannot be used to obtain an estimate of the variation in spore volume.

On the assumption of unit density (Buller 1909) the result corresponds to a spore mass of $(7.81 \pm 2.25) \times 10^{-11}$ gms. This is in general agreement with the results obtained by direct weighing.

Method (c). Interference Microscopy

It is possible to measure the density (in the mass/volume sense) of a small object by determining the retardation of a light wave passing through it. Dry rot spores proved quite suitable material on which to use the interference microscope but the interpretation of the results in terms of mass by this method is only valid if the material is more or less free of lipids. Thus it was necessary to have some knowledge of the chemical composition of the spores in this respect. For this reason, and because of the agreement between the results obtained by the methods already described, it was thought unnecessary to pursue the interference microscopy technique further.

Calculation of the Charge on a Mean Spore

Using the value of $(5.52 \pm 0.481) \times 10^{-11}$ gms. obtained for the $\frac{e}{m}$ experiment spores by direct weighing, the value determined for $\frac{e}{m}$ (245 e.s.u./gm.) gives a charge on the mean spore of $(1.35 \pm 0.12) \times 10^{-8}$ e.s.u.

Discussion

(a) Experimental Errors.

These are considered under the following headings:

(I) Determination of the peak of the $\frac{e}{m}$ distribution.

(II) Effect of finite slit width.

(III) Field distortion at top and bottom of the plates.

(IV) Changes in the state of the falling spores.

(V) Errors in measurement.

(VI) Measurement of mass.

(I) An analysis of the double peak distribution into its component parts shews that the overlap of these single distribution curves has no influence on the positions of the peaks. Thus we are justified in taking the separation of the observed peaks of the distribution as a measure of twice the mean displacement.

(II) The finite slit width may be expected to contribute to the width of the distribution. If the distribution be regarded as the sum of distributions of spores entering uniformly over the slit, then the correction to the variance will be given by Sheppard's adjustment (Weatherburn 1949). The value of Sheppard's adjustment in the experiment is (width of slit)^2 / 12 = $\frac{(0.07)^2}{12}$ = 4 x 10^{-4} sq. cm. The variance is 0.21^2 sq. cms. = 441 x 10^{-4} sq. cms. Thus the adjustment is only of the order of one per cent of the variance and may legitimately be neglected.

(III) The calculation of $\frac{e}{m}$ has been made on the assumption that the field is uniform within the apparatus. The field is, however, certainly distorted by the presence of the earthed outer box.

Near the slit end the field in the centre of the apparatus will be appreciably reduced by the presence of the plate containing the slit. Electrolytic trough measurements lead to the conclusion that within seven millimetres of the slit plate the field reaches 90% the value that it would have were the plates infinitely long, and that within one centimetre of the top it will be very close to this value. This reduction of the field in the first few m.m. of fall will lead to an underestimate of the value of $\frac{e}{m}$ by effectively reducing the length of the plates. Without very detailed calculations it is not possible to estimate the effect precisely, but it

cannot be greater than 5% and is unlikely to be greater than 2%. There will be a similar though much smaller effect at the bottom of the apparatus.

(IV) Buller's (l.c.) observations indicate that the spores may be expected to have dried out and be in equilibrium with the atmosphere falling with a steady terminal velocity before entering the apparatus.

(V) Measurements were made on the base plate with the mechanical stage of the microscope. Measurements of the apparatus were made with vernier calipers. The measurements of the potential difference between the plates weremade with a laboratory voltmeter. The errors inherent in all these measurements are negligible under the conditions of the experiment.

(VI) It is noted that no satisfactory measure has been obtained for variation in spore mass. It seems reasonable that in one sample, collected from one fruit body over a short period, the variation would not be very great.

However, over longer periods variation in spore size has been shewn to be considerable in Polyporus fomentarius (Bjørneker 1937). The considerable variation in spore sizes given in the literature is doubtless due to a real variation as well as to variation in the conditions in which the measurements were made.

(b) Charging Mechanism

The result obtained for the magnitude of the charge on the mean dry rot spore is of the same order as that obtained in the spray electrification of oil droplets of about the same size (Hopper and Laby l.c.). It is thought that there is no reason to suggest any mechanism other than the mechanical separation of spore from sterigma to explain the origin of the charges on the spores. The asymmetry in the distribution may possibly be related to a difference between the sterigmata and apiculae walls.

(c) Biological Importance

The possibility of spore charges being important in liberation from the fruit bodies of narrow pored species has been suggested by Ingold (1957). In such fungi the spore would tend to be attracted to the pore wall, provided it were not in the position of unstable equilibrium in the centre of the pore, and thus the chance of liberation would be reduced. However, charges of the same order of magnitude as that found for the dry rot spores would effect little horizontal displacement. Thus a spore, with a mass of 4×10^{-11} gms., a charge of 2×10^{-8} e.s.u. and 10^{-3} cms. (i.e. about 1 spore diameter) from the pore wall, would experience a horizontal force of $\frac{(2 \times 10^{-8})^2}{(1 \times 10^{-3})^2} = 4 \times 10^{-10}$ dynes from the induced charge. The gravitational force on such a spore would be $4 \times 10^{-11} \times g = 4 \times 10^{-8}$ dynes, i.e. one hundred times greater. It is also borne in mind that spores are normally discharged further than 10^{-3} cms. (see Part II) into the pore and thus the difference

in order between the two components will be even greater.

It thus seems unlikely that the charges play any important part in the escape of spores from fruit bodies, although the asymmetrical distribution of charge in the spore population must be accounted for in any theory of the mechanism of spore discharge.

Part II. Liberation from the Pore, with particular reference to *Polyporus betulinus* Fr.

The discussion of spore electrostatics (Part I) has indicated that electrical forces are unlikely to influence the movement of spores within pores to any appreciable extent. The influence on spore liberation of forces other than electrostatic ones remains to be discussed.

The trajectory of a basidiospore on discharge has been calculated by Buller (1909) from a knowledge of the maximum horizontal distance of discharge from a vertical hymenium, and the terminal velocity with which the spore falls towards the earth. Spores following this trajectory would first move horizontally a distance of up to about 0.2 mm and then, almost instantaneously, begin to fall vertically. Spores on emerging from the fruit body were seen to revolve in an irregular manner and long spores appeared to follow steep corkscrew paths. It was noted that as a spore fell it dried out and its velocity diminished. Buller also noted that even very slight air currents would disturb vertical spore fall. He concluded that, in the liberation from pore, gills etc., the spores followed this trajectory determined by the initial velocity of projection from the sterigmata and the gravitational attraction.

Ingold (1947) suggested that other forces in addition to gravity might be involved, as substantial spore deposits could be obtained from the collected fruit bodies of small-pored species without particular regard to their orientation. The results of Part I indicate that electrostatic forces are so small as to be negligible in this context.

Figure 1.

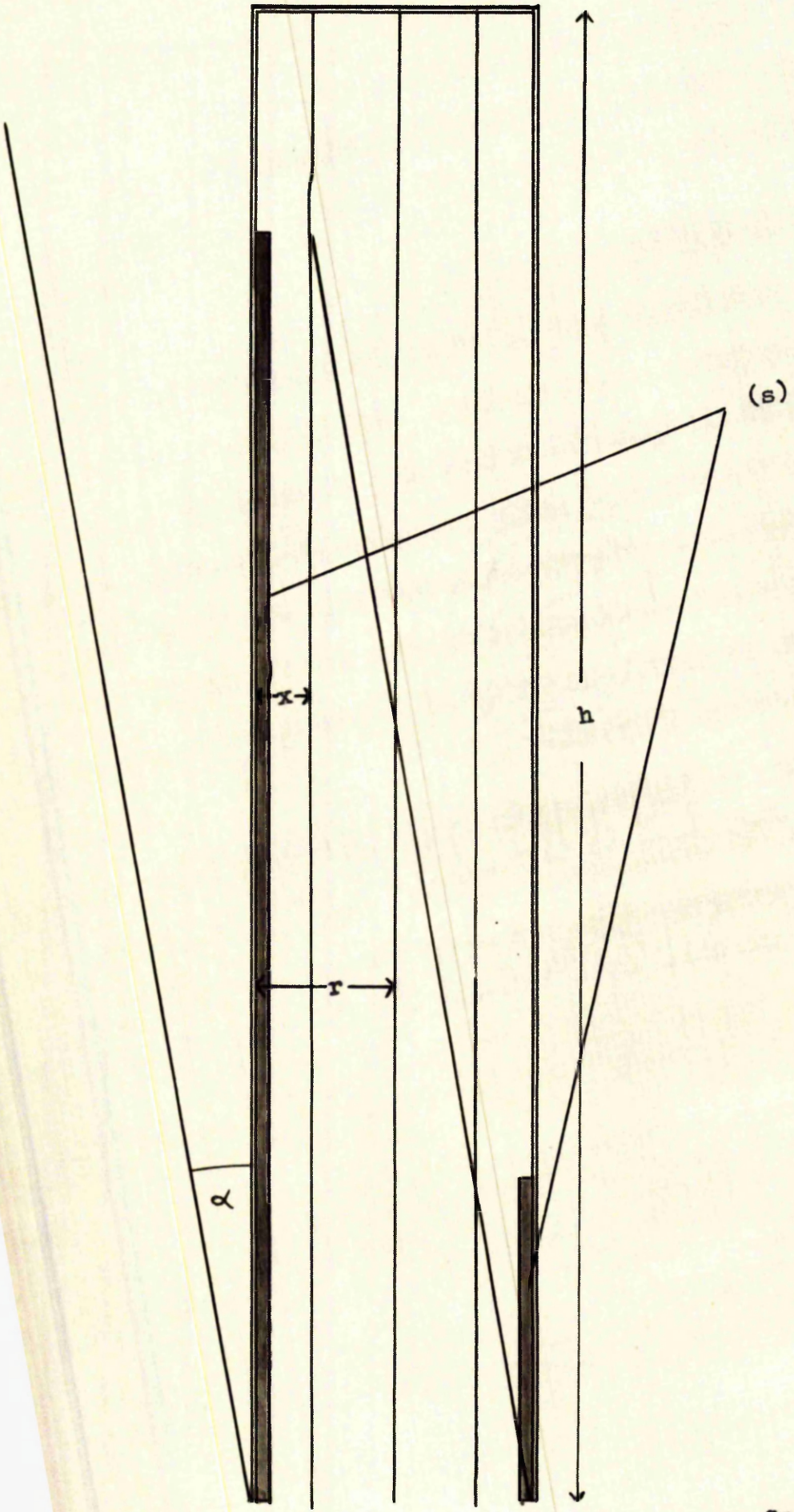


Diagram of Pore - See Text

The existence of other forces is investigated by comparing the diminution in liberation from a displaced pore with that expected from a geometrical model, in which the spores are supposed to follow the trajectory determined by Buller.

Theoretical Considerations

Consider a cylindrical pore of height (h) and radius (r) in which the hymenum covers the entire curved surface. Let the spores be initially discharged normal to the hymenium so that at a distance (x)* from the nearest portion of the pore wall their paths shew an instantaneous change in direction, further spore movement being vertical (see figure 1). (This trajectory is a good approximation to that derived in Buller's calculations in which he shewed the change in direction to be accomplished in only a few μ of vertical displacement.)

On displacement (α°) from the vertical, the surface area (s) of the hymenium from which spores having this prescribed trajectory can pass through the orifice is shown in Appendix $\breve{D}$ to be given by the following equations.

---

* It is to be noted that (x) is not the distance of discharge normal to the hymenium but the distance of the spore from the nearest part of the pore wall at the moment when spore fall becomes vertical. Its value therefore cannot exceed (r).

Case 1 where $x = o$

$$s = 4r^2 \cot \alpha \qquad \text{where } \tan \alpha \geqslant \frac{2r}{h}$$

$$\text{and } s = r \cot \alpha \left[2h \tan \alpha \sin^{-1} \sqrt{\frac{r^2 - \frac{h^2 \tan^2 \alpha}{4}}{r}} + 4 \left\{ r - \sqrt{r^2 - \frac{h^2 \tan^2 \alpha}{4}} \right\} \right]$$

$$\text{where } \tan \alpha \ll \frac{2r}{h}$$

Case 2 where $x = r$

$$s = 2\pi r^2 \cot \alpha \qquad \text{where } r \cot \alpha \leqslant h$$

$$\text{and } s = 2\pi r h \qquad \text{where } r \cot \alpha \geqslant h$$

Total surface area of the hymenium $= 2\pi r h$.

These equations cover the cases for the extreme values of (x) which must lie between zero and (r). The mathematical solution for intermediate values of (x) is of such complexity that it is not considered. However, it is obvious that the spore discharged into the centre of the pore will have the best chance of escape.

If the whole hymenial surface is sporulating at a uniform rate, the surface area of hymenium from which spores can escape (s) will be proportional to the number of spores liberated, in unit time. The surface area (s), expressed as a percentage of the total surface area, is thus equivalent to the percentage liberated of the total number of spores produced in unit time.

The applicability of these equations to any species must depend on the degree to which the pores conform to the assumptions made in their derivation.

Experimental

Polyporus betulinus Fr. was chosen for this investigation, being the most common small pored polypore in the district. All material was collected in Dunbartonshire (V.C.99). The form of the pores in this species is first considered. Experiments are then described in which the effect of tilting the known pores on spore liberation was measured.

1. The Pores of *Polyporus betulinus* Fr.

(a) Development of Pore Layer

Field observations made occasionally throughout the season on the fruit body population in local woods indicate that the pores continue growing during the life of the fruit body, a period of some months. Corner (1953) states that the pores of *Polystictus microcyclus* develop by a superficial meristem. It is not stated in his description of *Polyporus betulinus* in the same paper how pore growth takes place, but from the account he gives of the structure of the pore layer it is difficult to imagine other than superficial meristematic activity. Confirmation of superficial pore growth in *Polyporus betulinus* is obtained from the pores of fruit bodies growing on partially collapsed trees. These pores shew a change in direction of development which may reasonably be related to the time of collapse of the tree. The *lower* portions of such pores shew the normal vertical orientation. It is thus concluded that the pore layer in *P. betulinus* deepens by means of a superficial meristem.

Table I

Surface Density of Pores in Polyporus betulinus

Pores/sq mm.	Position	Source or Author
8.9	A	Milngavie
9.1	B See Fig. 2	Milngavie
8.0	C	Milngavie
12.3*	Centre	Milngavie
9.6	Centre	Milngavie
10.2	Centre	Milngavie
4-9	-	Murrill (1903)
9-16	-	Bourdot et Galzin (1927)
9-16	-	Lowe (1934)
9-16	-	Overholts (1953)

* Mean of determinations for 20, 6.25 sq. mm. areas.

(b) Number of Pores

Estimates of the surface density of pores were made for three fruit bodies. In two instances this was done by counting the number of spores in a substantial area (of the order of ½ a sq. cm.) and once by counting the number of spores in numerous much smaller areas. These results are given in Table I together with some values from the literature. It can be seen that these previous estimates are confirmed and it is concluded that the pore density in Polyporus betulinus is of the order of 10 pores/sq. mm.

(c) Shape of the Pores

Pore shape was investigated in an apparently typical fruit body collected on the third of February 1958.

The radial* pore walls, in this and other fruit bodies collected in the latter part of the season, tended to project below the tangential ones. This feature was not seen earlier in the winter, nor in the fruit bodies used in the experimental work described below. This irregularity of the undersurface, which appears to be a feature of old fruit bodies, is often mentioned in the literature; e.g. 'Orifices toothed' (Massee 1892), 'Mouths very irregular' (Murrill 1902), 'Occasionally becoming quite dentate' (Overholts 1953). This irregularity will doubtless affect the uniformity of spore discharge round the orifice to a slight extent.

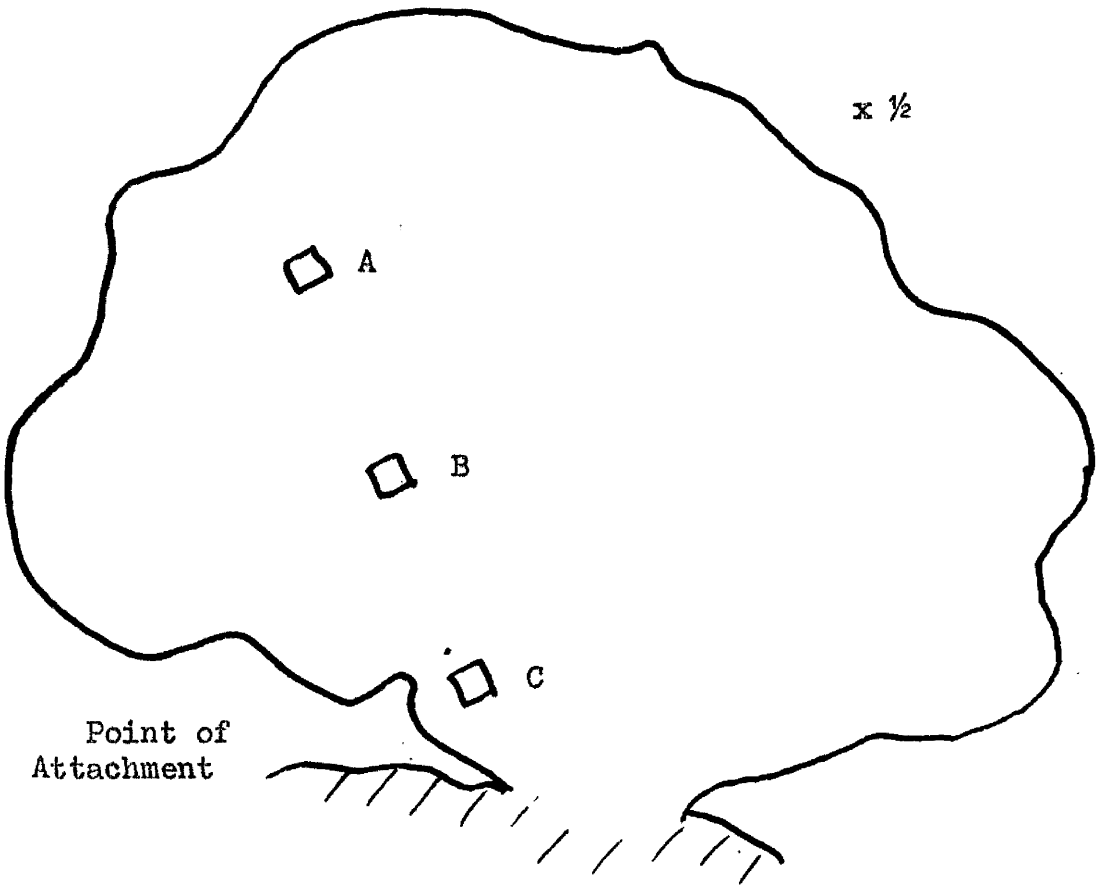
The degree to which the pores approximated to cylinders was

---

* In this part the term radial is used to describe the horizontal directions passing through the point of attachment and normal to the curved edge of the fruit body. Tangential refers to the direction in the horizontal plane normal to this.

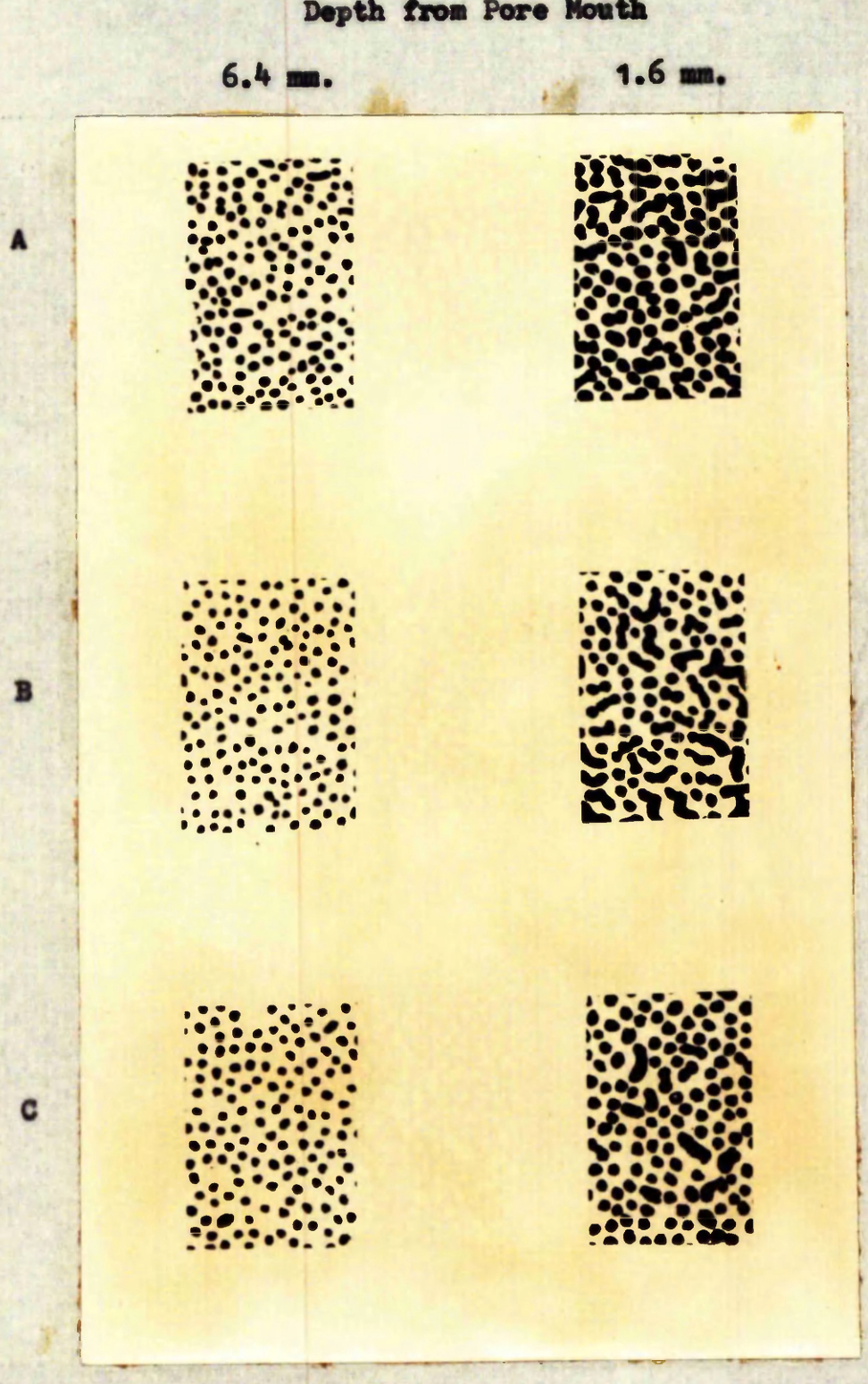
Figure 2.

Drawing of Polyporus betulinus fruit body shewing positions from which sections were cut.



For explanation see text.

Figure 3. Sections of the Pore Layer of Polyporus betulinus from Positions A, B and C.



x 7½

Table II

Variation in Pore Diameter in Polyporus betulinus

Depth from Mouth mm.	Mean Diameter mm.		
	A	B	C
0.8	(F4)	-	(F3)
1.2	(F4)	(F5)	(F3)
1.6	(F2)	(F5)	-
2.0	(F2)	(F5)	0.38
2.4	0.37	(F5)	0.38
2.8	0.43	(F5)	0.37
3.2	0.37	0.36	0.28
3.6	0.31	0.32	0.30
4.0	0.35	0.31	0.28
4.4	-	0.31	0.33
4.8	0.40	0.29	0.39
5.2	0.30	0.27	0.36
5.6	0.35	0.26	0.32
6.0	0.23(4)	0.31	0.32
6.4	-	0.29	0.38
6.8	-	0.26(4)	0.35
7.2	-	0.27(4)	0.36
7.4	0.23(4)	-	-
7.6		0.25(4)	0.30
8.0		0.22(2)	0.29
8.4		0.21(2)	0.31
8.8		0.21(2)	0.27
9.2		-	
Depth pores	7.44 mm.	9.2 mm.	9.0 mm.

(Fx) indicates x pores being fused.

(y) indicates that y pores were measured to obtain the mean where the number was not the full complement of 5.

investigated. Serial sections of the pore layer were cut, 0.4 mm. thick, with a freezing microtome from positions A, B and C (figure 2). These sections were mounted in glycerine and projected through a photographic enlarger on to printing paper. From each of the prints measurements of the radial and tangential diameters for five randomly selected pores in each series (A, B and C) were made. The sections close to the oriffices were not measured, as the irregularities of the walls described above resulted in their being incomplete and distorted. No attempt was made to estimate the diameters of individual pores where two or more had merged.

The sections 1.6 and 6.4 mm. from the pore mouths are shewn in figure 3 and the results are summarised in Table II. In all three cases there is a tendency for the pores to become narrower at the top, although within this general decrease there may be small local increases. The diameters at the top are of the order of 70% of those at the mouths. The pores in this, and not infrequently in other mature fruit bodies, are found to have merged in groups near the surface.

The ellipticity of the pores was investigated by a comparison of the radial and tangential diameters. The results are shewn in Table III. Analysis of these results shews that at:

position A radial diameter tangential ($\chi^2 = 6.61$)

" B " " " ($\chi^2 = 48.43$)

" C " " " ($\chi^2 = 3.52$)

Thus the pores are not necessarily round and at any particular place may shew a tendency to either tangential or radial elongation though the differences in diameter are very small (Table III).

Table III

Comparison of Radial and Tangential Diameters of Polyporus betulinus Pores

Part of Fruit body	Number of Pore	Number of times		
		r > t	r = t	r < t
A	1	6	2	2
	2	8	1	1
Mean r = 0.351 mm.	3	9	1	0
Mean t = 0.336 mm.	4	2	2	8
	5	9	1	9
Totals		34	7	15
B	1	0	1	8
	2	0	0	12
Mean r = 0.269 mm.	3	0	0	15
Mean t = 0.302 mm.	4	2	4	9
	5	0	0	12
Totals		2	5	56
C	1	13	4	3
	2	6	3	9
Mean r = 0.339 mm.	3	6	3	9
Mean t = 0.330 mm.	4	13	1	4
	5	12	1	7
Totals		50	12	32

t - diameter of pore measured tangentially to periphery of fruit body.

r - diameter normal to t.

Table IV

Pore Radius in Polyporus betulinus

Radius mm.	Position	Date	Source or Author
0.17*	A **	3rd Feb.	Milngavie
0.15*	B See fig. 2		
0.14*	C		
0.09	Measurements made of orifices or pores whose prints were counted (Section III)	18th Jan.	
0.08			
0.08			
0.09			
0.11			
0.08		15th Feb.	Coulport
0.08			
0.06			
0.08			
6.05	-	-	Massee (1892)
0.075-0.125	-	-	Bourdot et Galzin (1927)

* means based on measurement of 5 pores.

** measurements 4 mm. from orifice.

It is noted that obliqueness in sectioning might give such a conclusion, but in any one section pores can be found shewing elongation in opposite directions, and also in parallel serial sections the direction of elongation of individual pores can change.

(d) Radius of Pores

It has been pointed out above that the pores are sometimes slightly elliptical, but the differences between the major and minor axes are so slight that a single measurement for radius is given in Table IV. No measurements of pore diameter or radius other than those given in Table IV have been found in the literature, the pores usually being described as 'very small' or 'minute'. The value of c. 0.5 mm. given by Massee is so different from the other values that it will be neglected in this discussion.

It is concluded that the pore radius in this species has a range of about 0.06 to 0.16 mm..

(e) Depth of Pores

The depth of the pore layer was measured in several mature fruit bodies. These measurements are shewn in Table V together with some values from the literature. It is noted, from the measurements made at different positions on the fruit body collected on the 3rd of February and confirmed by other observations made in the field, that the pore layer is thinner towards the edges of the fruit body.

It is concluded that the depth of the pore layer has a range from about 3 to 10 mm.

Table V

Pore Depth in Polyporus betulinus

Depth mm.	Part of Fruit Body	Date	Source or Author
7.4	A	3rd Feb.	Milngavie
9.2	B See fig. 2		
9.0	C		
7.0	Centre	29th Nov.	Milngavie
7.0	Between centre and point attachment	18th Jan.	Milngavie *
6.0		15th Feb.	Coulport
5-10	-	-	Mayr (1884)
5	-	-	Murrill (1903)
2-8	-	-	Rea (1922)
3-9	-	-	Bourdot et Galzin (1927)
2-8	-	-	Lindau (1928)
3-9	-	-	Lowe (1934)
2-8	-	-	Overholts (1953)

* Fruit bodies used in experiments described in Section II.

(f) Extent of hymenium

The material used for these observations was that used in the experimental work described below.

Following the method described by Buller (1909) sections of the pore layer, about 1 mm. thick, from different depths were placed on microscope slides in a humid atmosphere and allowed to sporulate for some minutes. Sections from the bottom, middle and top of the pore layer produced similar numbers of spores in unit time.

Hand sections of other fruit bodies shewed mature basidia throughout the pores.

The conclusion is that there is a uniformly active hymenium covering the total area of the pore walls. Thus the number of spores produced by any part of a pore's hymenium will be proportional to the area of that part.

It is uncertain whether there is a well developed hymenium over the top of the pores, but this point is trivial since the area of such a hymenium in Polyporus betulinus would only be of the order of a two hundredth of that lining the walls. This area can therefore be neglected.

From the investigations described in (c) and (f) above it is concluded that the pores in Polyporus betulinus approximate sufficiently to cylinders, covered by a uniformly sporulating hymenium on their curved surfaces, for the equations given above to be applicable.

Photograph 1.

Under surface of Coulport fruit body of Polyporus betulinus

x 5

Photograph 2

Fruit body of Polyporus betulinus on tilting table

$\times \frac{1}{10}$

2. Effect on Spore Liberation of Displacing Polyporus betulinus Pores

Method

The observations were made on a fruit body from Coulport on the 15th of February.

The orientation of the fruit body on the tree was recorded by placing a three legged spirit level on the upper surface in a position in which the bubble was centred and marking the positions of the legs with Indian ink.

A short section of the trunk bearing the fruit body was then cut from the tree. The undersurface of the fruit body was marked with small glass reference pins (photograph 1) and long surgical pins inserted into both fruit body and trunk to support a microscope slide immediately beneath the undersurface (photograph 2).

Photograph 2 also shews the tilting table on which the fruit body was displaced. This device enabled displacement to be made in two planes at right angles.

Immediately after it had been cut from the tree the fruit body was set up on the tilting table near to the base of the tree and brought back to its previous orientation. A slide was then exposed for ten minutes. The morning was cold (air temperature 38°F) and windy, and the fruit body was covered with a biscuit tin to prevent draughts. As the spore deposit was slight (about 10 spores/pore) the remainder of the experiment was continued indoors, after allowing several hours for the fruit body to come into equilibrium with the warmer atmosphere (c. 55°F).

Spore deposits were obtained for a series of displacements about an axis

Photograph 3

Examples of spore deposits for different displacements

x 2

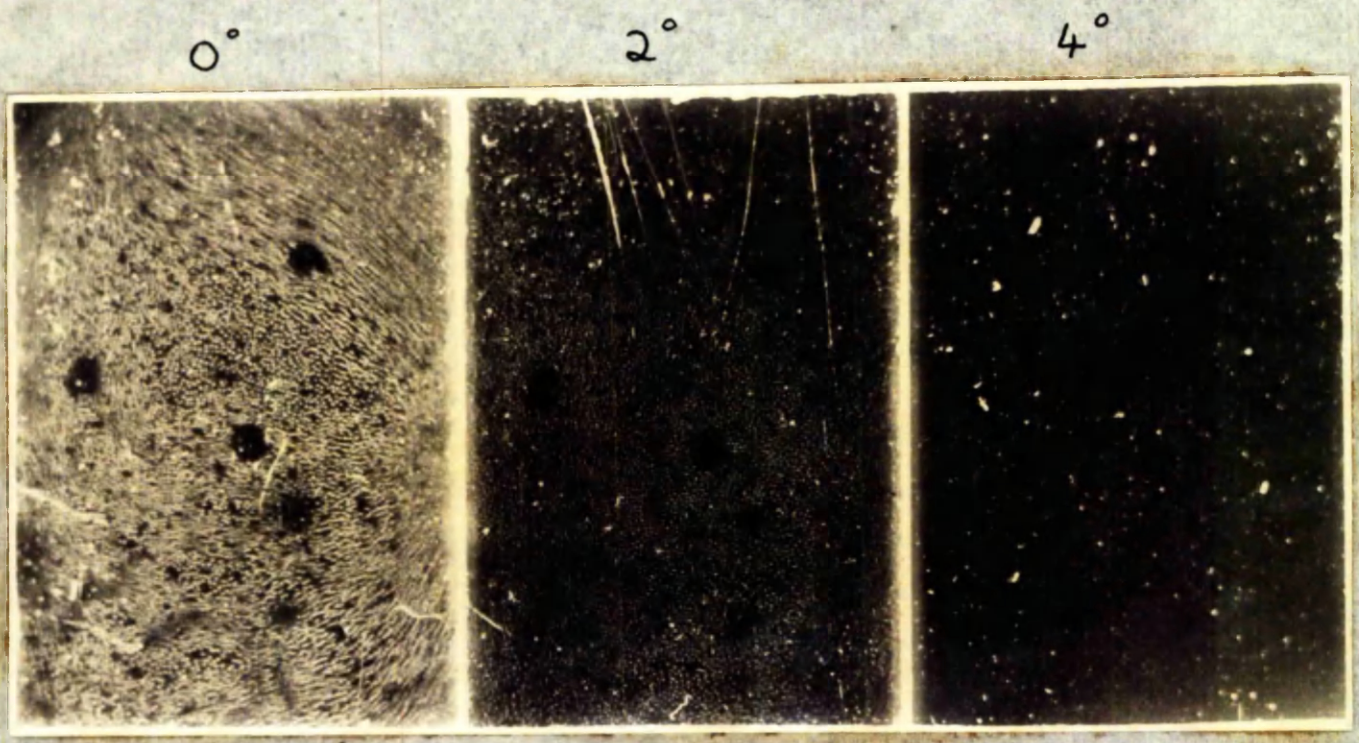


Figure 4.

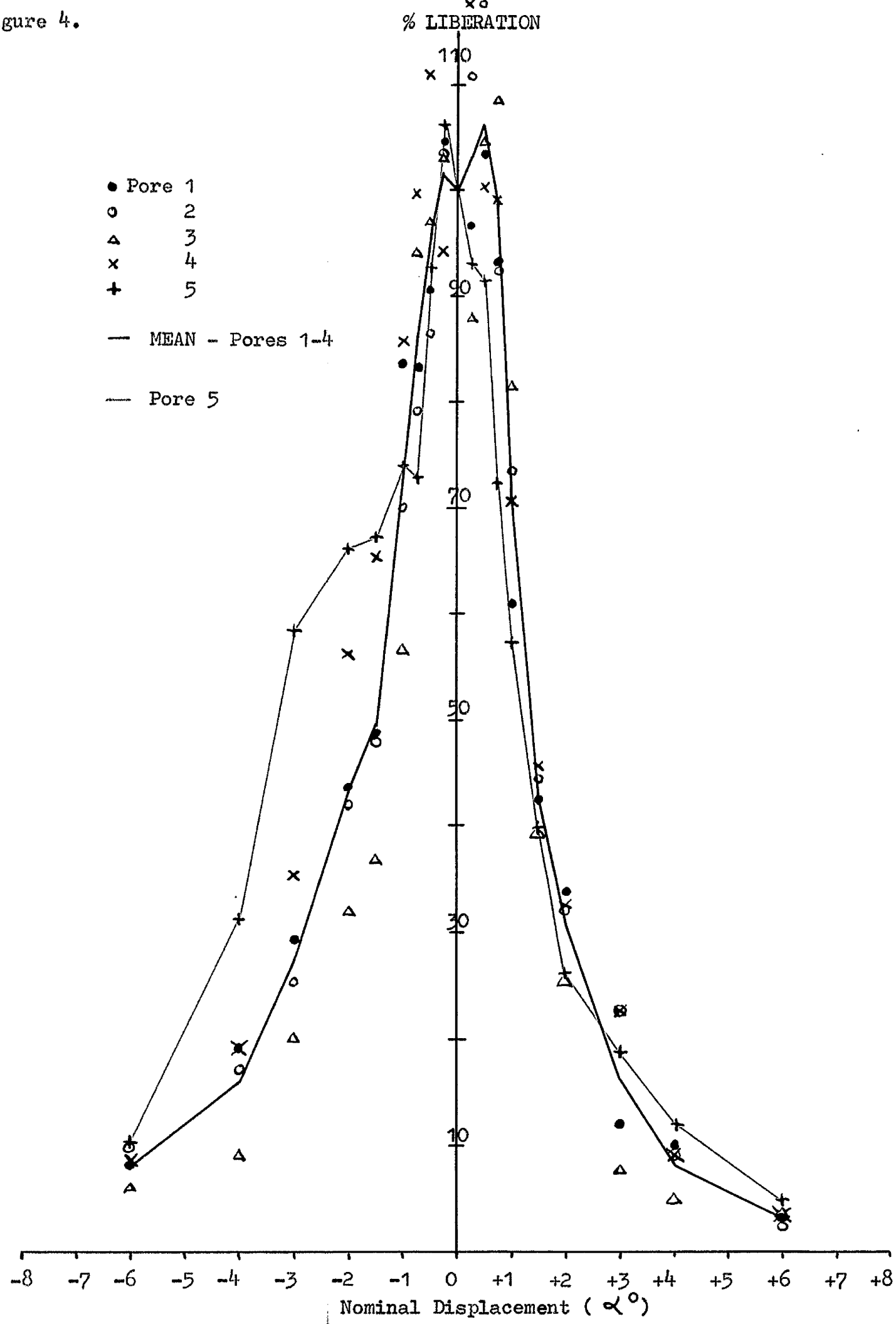
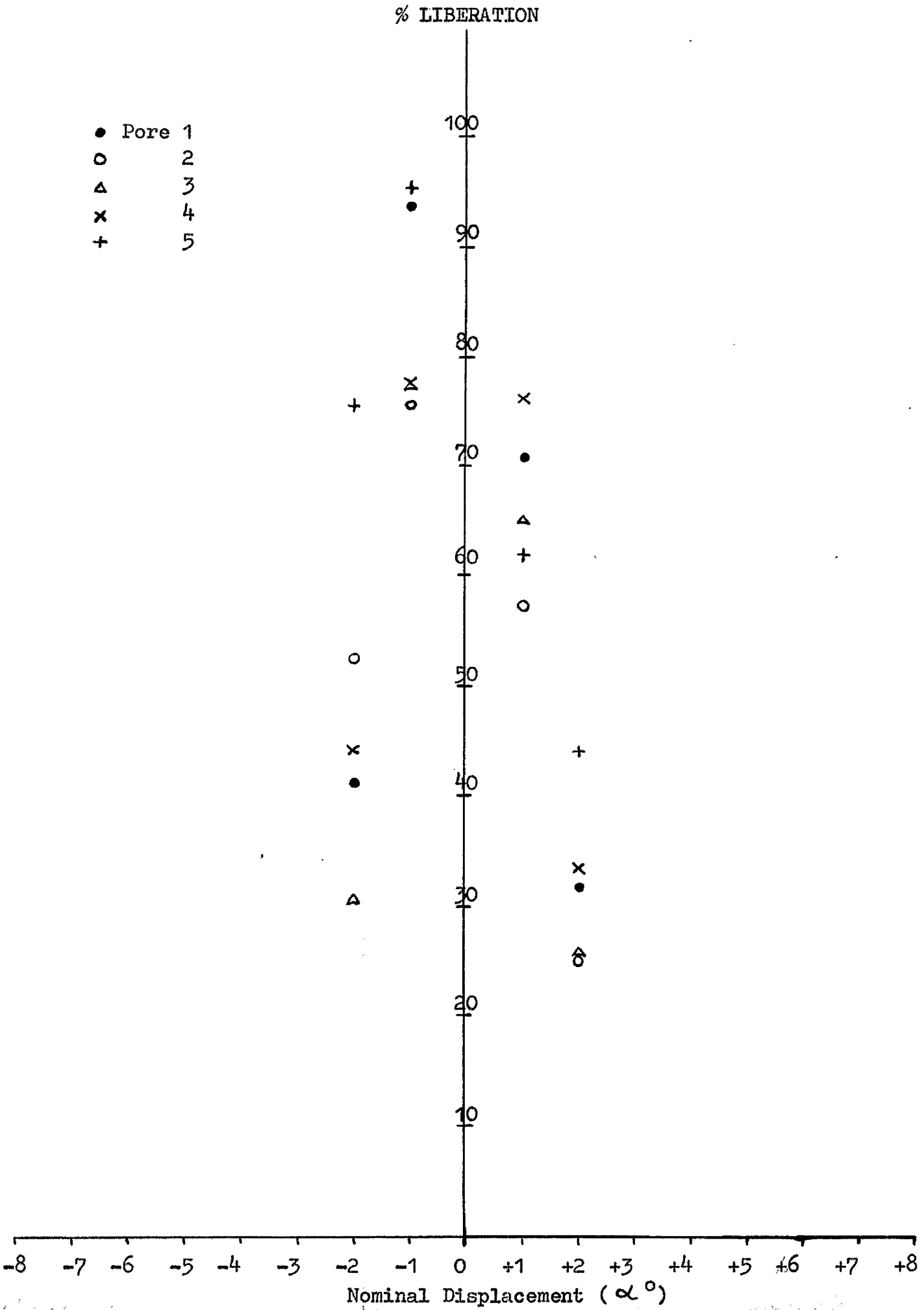

% LIBERATION
110
90
70
50
30
10
Pore 1
2
3
4
5
MEAN - Pores 1-4
Pore 5
-8 -7 -6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8
Nominal Displacement (∝°)

Figure 5.


% LIBERATION
● Pore 1
○ 2
△ 3
× 4
+ 5
100
90
80
70
60
50
40
30
20
10
-8 -7 -6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8
Nominal Displacement (α°)

tangential to the centre of the edge of the fruit body. Each slide was exposed for three minutes, the fruit body being covered with the biscuit tin to prevent draughts during the exposure. The displacements were measured with a Royal Artillery field clinometer. A deposit for the normal orientation was obtained before and after each exposure for a particular displacement. This gave a standard which allowed for variation in the sporulation rate, and with which the deposits for particular displacements could be compared. A shorter series of displacements was made at right angles to this first series.

Five deposits from individual pores, easily recognisable on each slide, were selected. For each of these the total number of spores in each pore's deposit was counted.

Results

Photograph 3 shows examples of spore prints for displacements of 0°, 2° and 4°.

The counts for the various displacements are shown in Table VI, together with the means of the pairs of counts made on the deposits obtained for the normal orientation immediately before and after each displacement. Table VII shows the counts for particular displacements expressed as a percentage of those for the normal orientation.

These results for the two series of displacements are shown graphically in figures 4 and 5. It can be seen from these figures that, while in figure 4 the maximum spore liberation occurs at the nominally zero displacement, in figure 5 the maximum occurs at about $-\frac{1}{2}^{\circ}$. This suggests

Table VI

Counts of deposits at different displacements

Main Series

Nominal displ.	Pore number 1		2		3		4		5	
	C*	Count	C*	Count	C*	Count	C*	Count	C*	Count
+ ¼°	119	115	101	112	100	88	95.5	112	107.5	100
- ¼°	287.5	301	281.5	291	227	234	220	207	174	185
+ ½°	122.5	127	112	131	88	92	104.5	105	111.5	102
- ½°	309	280	285.5	247	218	211	200	222	191	177
+ ¾°	124.5	116	138	128	98.5	107	108	107	123	89
- ¾°	297	247	305.5	242	220	185	204.5	202	189.5	138
+ 1°	223.5	136	232.5	171	163	133	180.5	128	197	113
- 1°	265	221	288.5	202	228	129	213	183	177	131
+ 1½°	264	112	263	117	189	74	208	95	194	77
- 1½°	283	138	283	136	236.5	87	198.5	130	168	113
+ 2°	260.5	88	245	79	200.5	51	203	66	187	49
- 2°	288	126	283.5	119	222	71	197.5	111	158.5	105
+ 3°	236.5	32	260	59	221.5	17	177.5	40	170	32
- 3°	283.5	83	281	71	214	43	200.5	71	126.5	74
+ 4°	247	25	280	26	225	11	207.5	19	162.5	21
- 4°	291.5	56	280.5	48	220.5	20	213	41	121.5	38
+ 6°	272.5	8**	286.5	8**	228.5	8**	235.5	8**	163.5	8**
- 6°	283.5	23	279	28	232	14	224	19	135.5	14

Minor Series - displacements at right angles to Main Series

Nominal displ.	Pore number 1		2		3		4		5	
	C*	Count	C*	Count	C*	Count	C*	Count	C*	Count
+ 1°	273	193	285.5	164	248.5	162	222	169	143.5	89
- 1°	268	251	300.5	227	257.5	199	235	182	158.5	151
+ 2°	298	95	298.5	75	259	67	248.5	83	171	75
- 2°	302.5	125	282	148	263.5	81	253	112	173.5	131

* C = mean count at normal orientation

** = estimate, pores not clearly distinguishable

Table VII

Effect of displacement on spore liberation

Main Series

Displacement		% Nominal zero displacement value					Mean
Nominal	Corrected	Pore 1	Pore 2	Pore 3	Pore 4	Pore 5	percentage
0°	0.50°	100	100	100	100	100	100
+ ¼°	+ 0.56°	96.6	110.9	88.0	117.3	93.0	101.2
- ¼°	- 0.56°	104.7	103.4	103.1	94.1	106.3	102.3
+ ½°	+ 0.70°	103.7	117.0	104.5	100.5	91.5	103.4
- ½°	- 0.70°	90.6	86.5	96.8	111.0	92.7	94.5
+ ¾°	+ 0.90°	93.2	92.8	108.6	99.1	72.4	93.2
- ¾°	- 0.90°	83.2	79.2	84.1	99.8	72.8	83.8
+ 1°	+ 1.12°	60.9	73.5	81.6	70.9	57.4	68.9
- 1°	- 1.12°	83.4	70.0	56.6	85.9	74.0	74.0
+ 1⅛°	+ 1.58°	42.4	44.5	39.2	45.7	39.7	42.3
- 1⅛°	- 1.58°	48.8	48.1	36.8	65.5	67.3	53.3
+ 2°	+ 2.06°	33.8	32.2	25.4	32.5	26.2	30.0
- 2°	- 2.06°	43.8	42.0	32.0	56.2	66.2	48.0
+ 3°	+ 3.04°	12.1	22.7	7.7	22.5	18.8	16.8
- 3°	- 3.04°	29.3	25.3	20.1	35.4	58.5	33.7
+ 4°	+ 4.03°	10.1	9.3	4.9	9.2	12.9	9.3
- 4°	- 4.03°	19.2	17.1	9.1	19.2	31.3	19.2
+ 6°	+ 6.02°	2.9	2.8	3.5	3.4	4.9	3.5
- 6°	- 6.02°	8.1	10.0	6.0	8.5	10.3	8.6

Minor Series - displacements at right angles to main series

Displacement		% Nominal zero displacement value					Mean
Nominal	Corrected	Pore 1	Pore 2	Pore 3	Pore 4	Pore 5	percentage
+ 1°	+ 1.5°	70.7	57.4	65.2	76.1	62.0	66.3
- 1°	- 0.5°	93.7	75.6	77.3	77.4	95.3	83.9
+ 2°	+ 2.5°	31.9	25.1	25.9	33.4	43.9	32.0
- 2°	- 1.5°	41.3	52.5	30.7	44.3	75.5	48.9

Figure 6.

DRAWINGS OF THE ORIFICES OF THE PORES
USED TO OBTAIN THE DATA SHEWN GRAPHICALLY

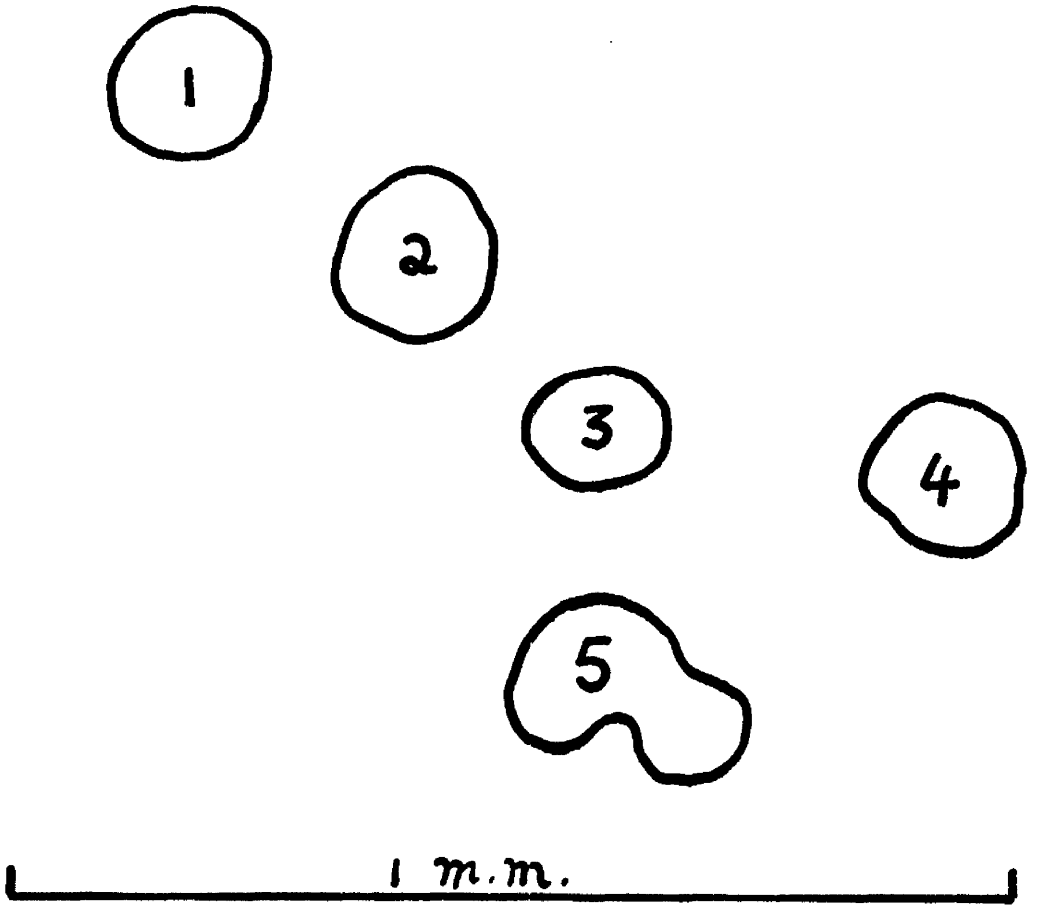


Figure 7.

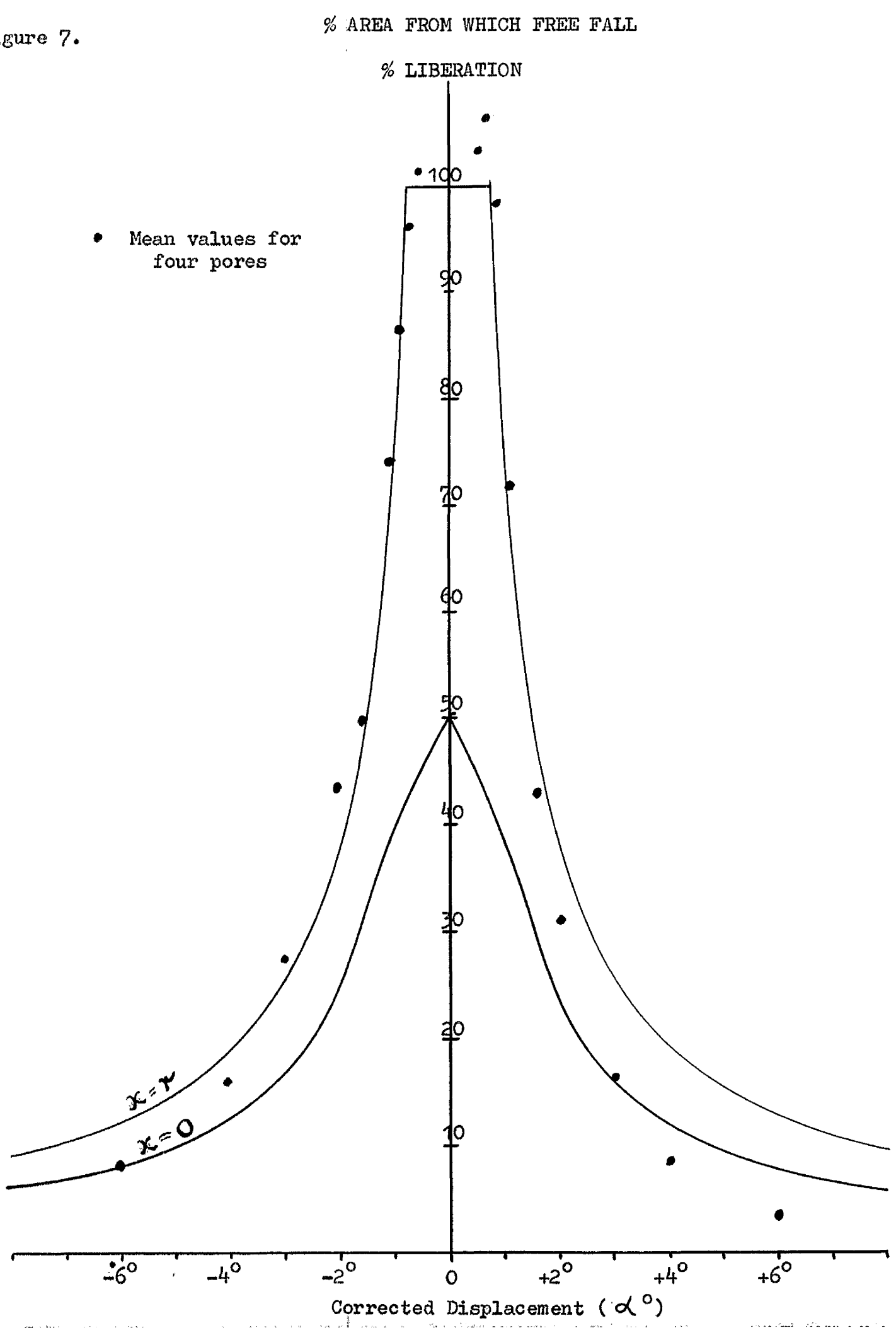

% AREA FROM WHICH FREE FALL
% LIBERATION
100
90
80
70
60
50
40
30
20
10
• Mean values for four pores
x=4
x=0
-6°
-4°
-2°
0
+2°
+4°
+6°
Corrected Displacement (α°)

Figure 8.

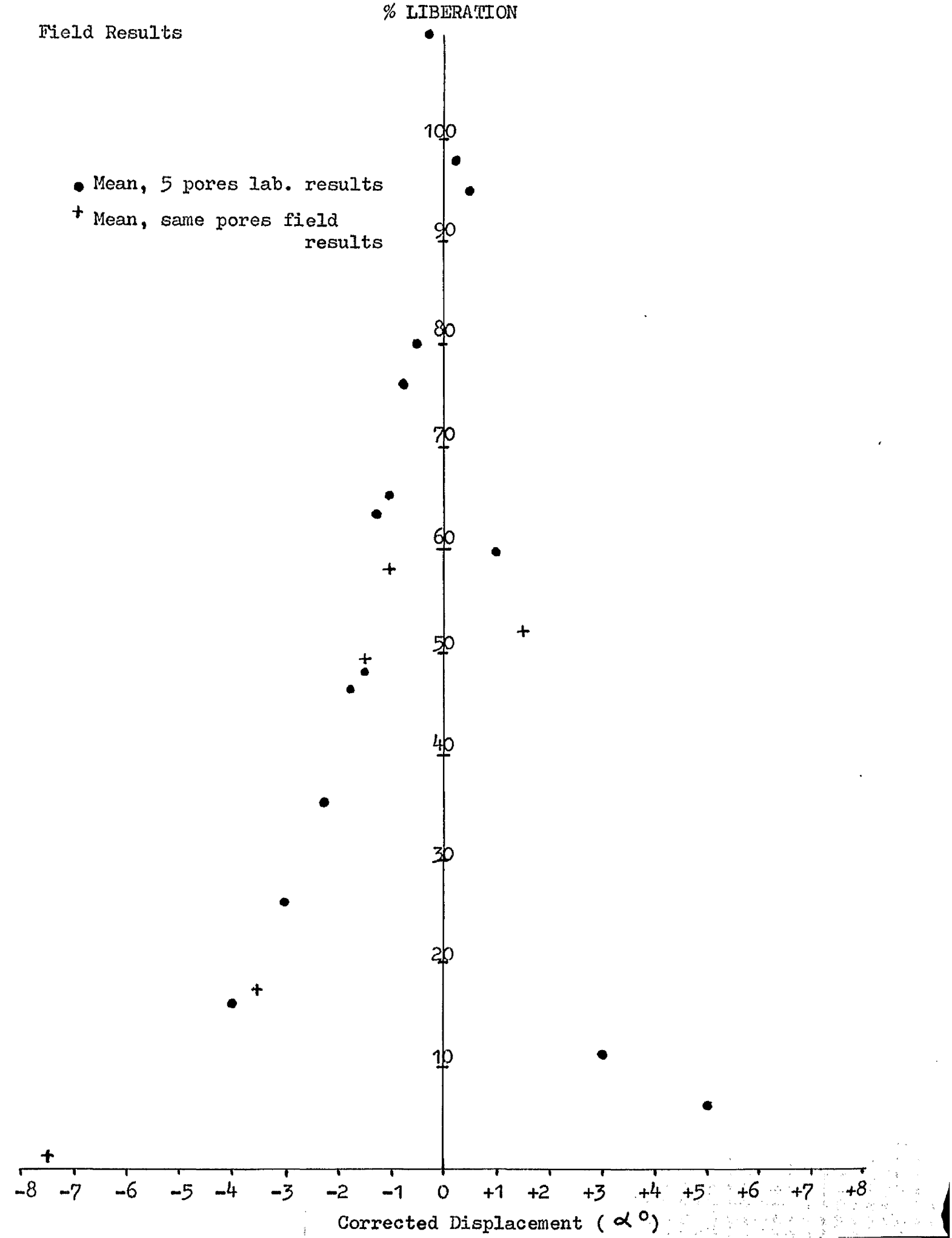
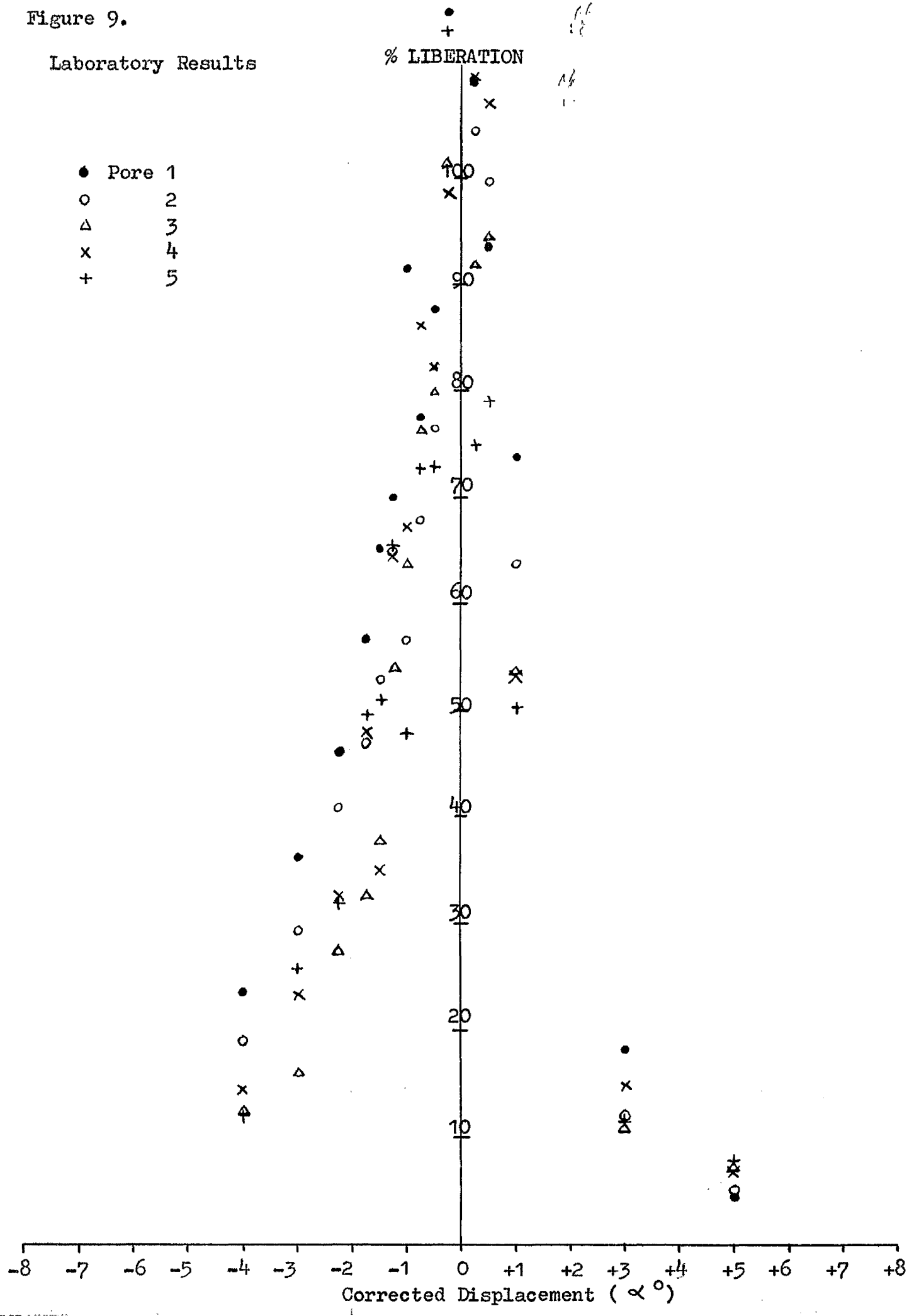

% LIBERATION
Field Results
● Mean, 5 pores lab. results
+ Mean, same pores field results
100
90
80
70
60
50
40
30
20
10
-8 -7 -6 -5 -4 -3 -2 -1 0 +1 +2 +3 +4 +5 +6 +7 +8
Corrected Displacement (α°)

Figure 9.

Laboratory Results



that maximum liberation shewn in figure 4 is not that for the pores in their normal orientation, but that obtained on a displacement of one half degree in the other plane. On this basis the values measured with the clinometer have been corrected (column 2 Table VII).

The drawing of the relevant pore orifices (figure 6) shews pore 5 to have an abnormal shape. The mean percentage spore liberation for the other four pores compared with that for this irregular pore is shewn in figures 4 and 7.

A preliminary experiment was carried out with a fruit body from Milngavie. The results (figures 8 and 9) for both field and laboratory experiments with this fruit body agree reasonably well with those described above. However, the method of levelling was less precise than that used in the experiment with the Coulport fruit body and thus the results do not warrant close analysis. The five pores whose deposits were counted all had typical orifices.

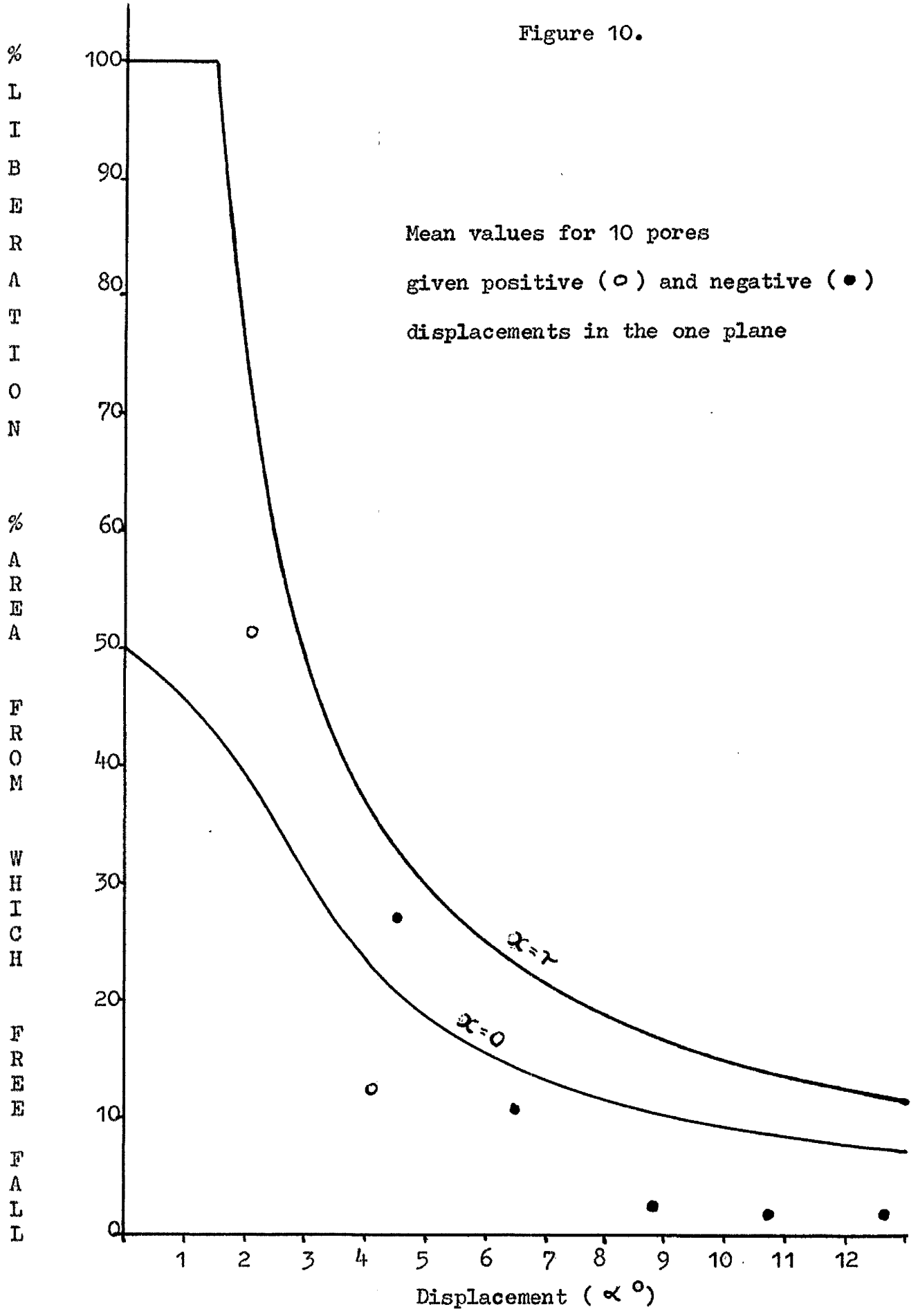
It appears from the results of these experiments that a displacement of about 1½° is sufficient to reduce spore liberation by 50%.

Supplementary Observations on displacing Boletus edulis (Bull.) Fr. Pores

Essentially the same technique as that already described for the Polyporus betulinus experiments was used. However, the method of recording the normal orientation using the clinometer instead of the spirit level together with a rather make-shift tilting table make the experimental technique rather less satisfactory. The experiment was carried out in the field (Balloch Park, Dunbartonshire).

The results are summarised in figure 10. It appears that a displacement

Figure 10.


% LIBERATION % AREA FROM WHICH FREE FALL
100
90
80
70
60
50
40
30
20
10
0
1
2
3
4
5
6
7
8
9
10
11
12
Displacement (∝ °)
Mean values for 10 pores
given positive (o) and negative (•)
displacements in the one plane
x=1
x=0

of about 2° will reduce spore liberation by 50%.

Comparison of Experimental Results with Theoretical Prediction

The relevant values of (r) and (h) for the fruit body used in the experimental work with Polyporus betulinus are shewn in Tables IV and V. The solutions of the equations for r = 0.08 mm and h = 6 mm are shewn in figure 7. It can be seen from this figure that the two curves (for x = o and x = r) lie fairly close together except for very small values of (α).

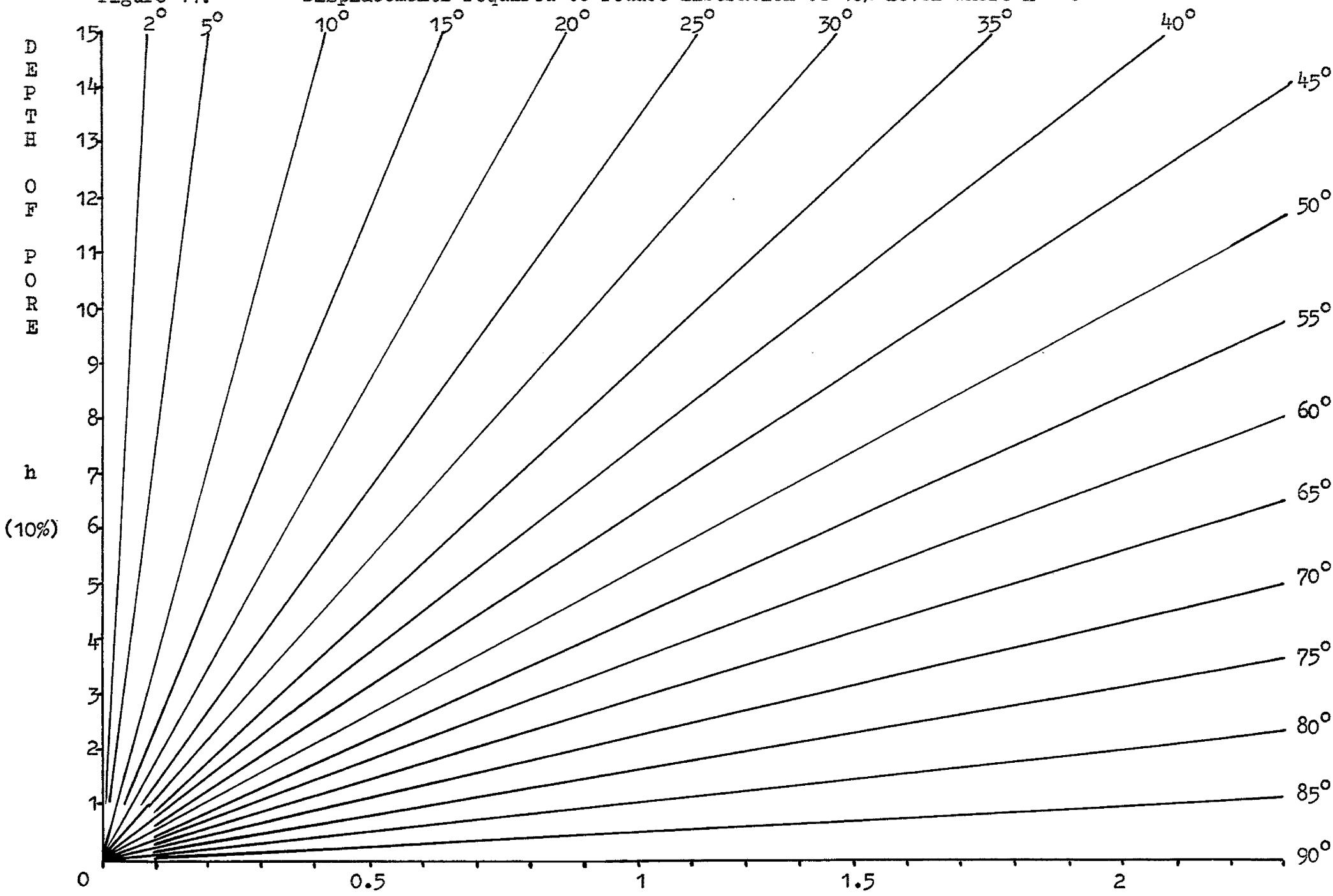
The comparison with the experimental values for diminution in spore liberation also shewn in figure 7 shews that these are in close agreement with the curve for x = r where the displacement is small ($< 2^{\circ}$), though for greater displacements the experimental values fall off rather more rapidly than the theoretical ones.

Observations made on the fruit bodies used in the experimental work, during the investigations of the extent of the hymenium (1(f) above), indicated that the spores tended to be discharged at least a third, or not more than two thirds, of the way across the pore, i.e. x is at least $\frac{2}{3}$ r or c. 0.05 mm or more. Thus it is probably better to compare the experimental results with the curve for x = r.

The solutions of the equations (for x = r and x = o) for values typical of the Boletus pores are shewn in figure 10. These curves have a similar form to the experimental one, although for the greater displacements the experimental values fall off more rapidly than the theoretical ones.

The discrepancy between the theoretical and experimental results for the

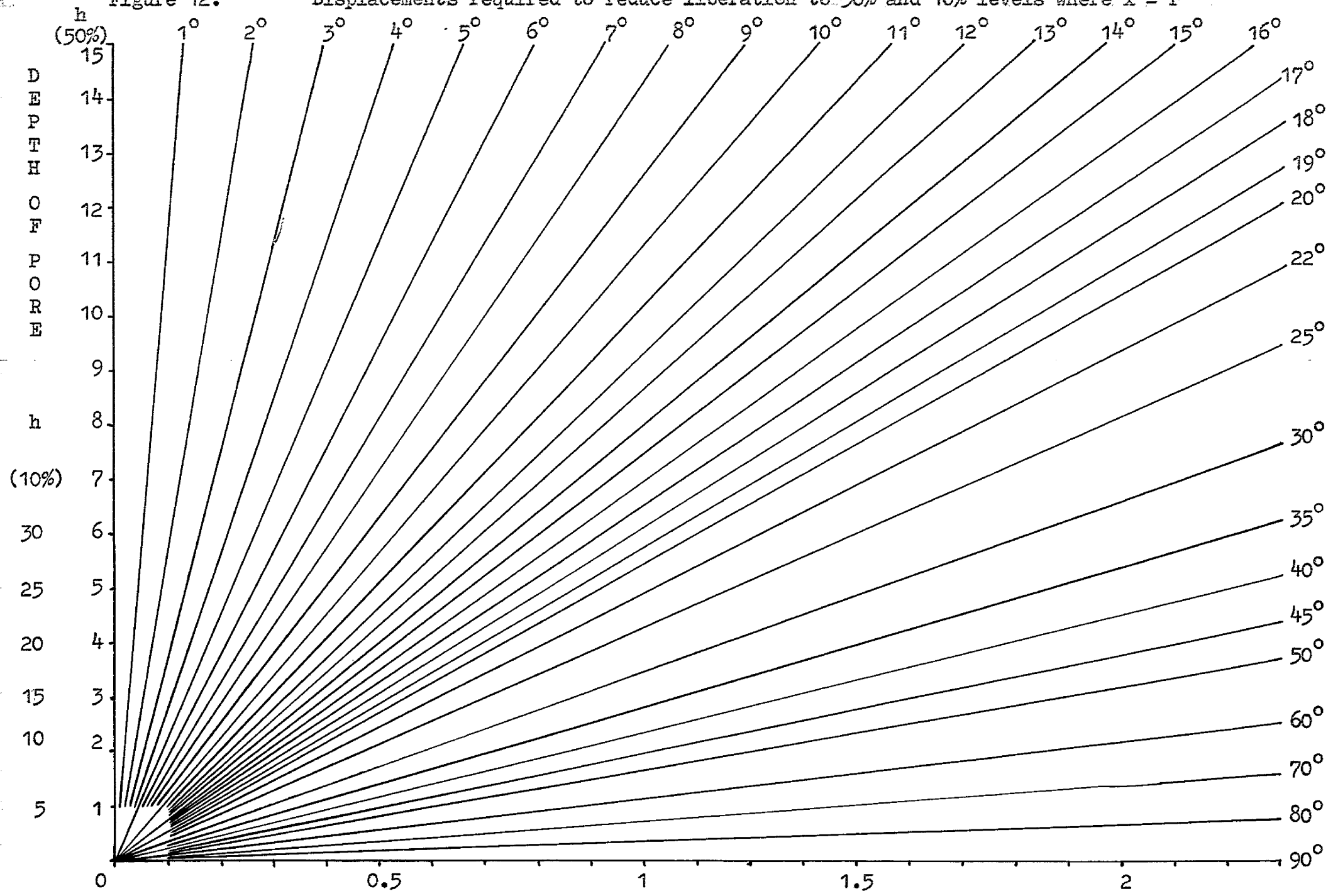
Figure 11. Displacements required to reduce liberation to 10% level where x = o



Pore radius in mm.

This figure is only applicable where $\alpha > 2^{\circ}$.

Figure 12. Displacements required to reduce liberation to 50% and 10% levels where x = r



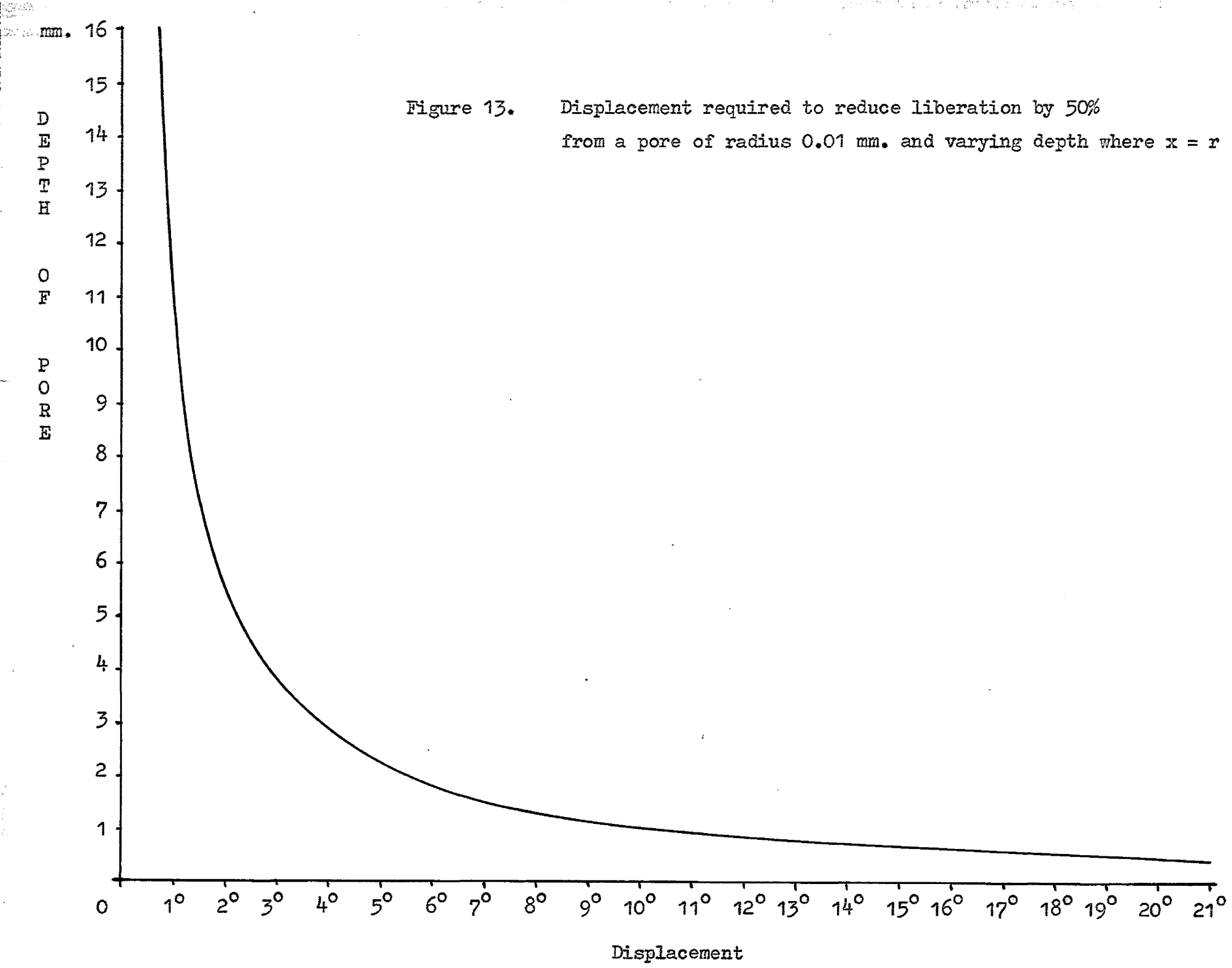


Figure 13. Displacement required to reduce liberation by 50% from a pore of radius 0.01 mm. and varying depth where x = r

greater displacements is possibly attributable to the increase in relative importance of the small immature growing zone at the pore mouths. The limits of this zone are not known precisely but it must be very small (probably much less than 1 mm) as sporulation throughout the pore as a whole has been shewn to be more or less uniform.

If the equations can be applied to other pored fungi whose fruit bodies fulfil the assumptions made in their derivation, the displacements expected to give reductions in spore liberation to the 50% and 10% levels can be determined from figures 11 and 12. Figure 11 shews the conditions for a reduction in spore liberation to 10% for the case where $x = o$. The 50% value for $x = o$ is achieved immediately ($\propto$) becomes greater than zero. In figure 11 where the pore dimensions are such as indicate a reduction in liberation to 10% for a displacement of less than 2^{o}, the precise displacement cannot be determined from the figure.

Figure 13 shews a calculation of the effect of increasing pore depth in Polyporus betulinus on spore liberation. In this species this calculation is of practical interest in that sporulation starts at the very beginning of pore development (Macdonald 1937) and continues throughout the life of the fruit body. It can be seen that after the pores reach a few millimetres subsequent growth makes relatively little difference to the liberation on displacement.

Conclusion and Biological Implications

The above comparison of theoretical and experimental results for Polyporus betulinus indicates that the diminution in spore liberation on displacement can be interpretated solely in terms of Buller's spore trajectory, it being unnecessary to postulate any other external forces for its explanation.

It is concluded that P. betulinus liberates its spores in such a way that the number of spores liberated on displacement is near the maximum possible if the trajectory is determined by the initial velocity of projection and the gravitational force on the spore.

The evidence from the Boletus work is consistent with interpreting the spore liberation mechanism in that species in terms of Buller's trajectory alone. However, the work on this species can only be regarded as supplementary evidence as no detailed investigation as to the form of the pores has been made.

It is clear from the work described that efficient liberation from long narrow pores demands a rigid form and stable substrate for the fruit body throughout its life. Despite this many of the longest lived species (e.g. Fomes) have long narrow pores. For example, Ingold (l.c.) found that in the fruit body of Ganoderma applanatum, the pores are 0.05 mm in radius and say 30 mm deep. The fruit body requires, according to the equation, a displacement of less than 0.2° to reduce liberation to 50% and less than 1° to reduce it to 10%. Many long lived, deep pored, species are of firm construction (see Corner l.c. on Polyporus betulinus), but despite this neither in P. betulinus nor Ganoderma applanatum has the ultimate reduction

in pore diameter, that is, to the distance of horizontal spore discharge, been achieved. A safety margin exists enabling liberation from the whole pore on very slight displacement.

The pore system offers a very great increase in hymenial surface (Buller 1909). Figures given above indicate that this in Polyporus betulinus is of the order of 40 times the area of the undersurface and Buller (1922) has obtained a value of 493 times for Ganoderma applanatum.

Many deep narrow pored species are long lived and in such instances the pore system may provide a hymenium protected from adverse and varying weather conditions, though it is noted that some Thelephoraceae can survive for a long time without any such protection.

The pore system in practice, at least in the case of Polyporus betulinus, appears to be very satisfactory both as regards the enormous numbers of spores readily obtained in the field and the extensive distribution of the species.

Part III. Variation in the Sporulation Rate of Trametes gibbosa Fr.

Diurnal sporulation rhythms have been recorded in the Phycomycetes and Ascomycetes.

The diurnal sporangiophore formation in Pilobolus has been known for over a hundred years (McVicker 1942) and has been demonstrated to be an endogenous rhythm controlled primarily by light but also by temperature (Schmidle 1951; Uebelmesser 1954).

In the Ascomycetes diurnal sporulation rhythms are apparently quite widespread (Table I). In Daldinia concentrica Ingold and Cox (1955) shewed that by halving the light and dark periods from 12 to 6 hours a second maximum in sporulation rate could be induced, which, unlike that of the normal rhythm, did not reappear in subsequent continuous darkness. They concluded that there was an endogenous rhythm present controlled by light and possibly by temperature. Light appears to be the most important controlling factor in cases where a 24 hour rhythm is involved.

Ingold and Dring (1957) shewed the rhythm in Sordaria fimicola to be non endogenous. Other cases await investigation.

In the Basidiomycetes there are no records of diurnal rhythms, although other irregularities in sporulation rate have been reported.

Von Schrenk (1900) stated that the spores of Polyporus schweinitzii came off at intervals 'as if they were discharged by some force acting within the tubes'.

Table I

Some examples of 24 hr. Sporulation Rhythms in the Ascomycetes

Species	Nature	Authority
Ascobolus immersus	diurnal	Buller, 1909
Podospora curvula	"	Ingold, 1933
Sporomia intermedia	"	" "
Nectria cinnabarina	"	" "
Hypoxylon fuscum	nocturnal	" "
Melanomma sp.	"	" "
Daldinia concentrica	"	Ingold, 1947
Epichloe typhina	diurnal	Ingold, 1948
Sordaria fimicola	"	Ingold and Dring, 1957
Erysiphe polygoni	"	Yarwood, 1936
Taphrina deformans	"	" 1941
Pseudoperonspora viticola	nocturnal	" 1937*
Peronspora destructor	"	" "
Plasmophora viticola	"	" "
Bremia lactucae	"	" "

* References to earlier observations on the downy mildews are given in this paper.

Banker (1910) observed the spores of Steccherinum septentrionale escaping in puffs of 10 to 15 seconds duration at two to three minute intervals. These puffs of spores were observed to escape from different parts of the fruit body at different times.

Buller (1909) made observations on Polyporus squamosus and other species from which he concluded that spore liberation was an uninterrupted process over long periods of time. He considered Banker's observations to be mistaken (Buller 1922).

Buller's investigations led him to classify the Agaricaceae into the Inaequihymeniferous (Coprinus) and the Aequihymeniferous (non Coprinus) types (Buller 1922, 1924). In the Inaequihymeniferous type the basidia mature successively from the gill edge. In the Aequihymeniferous type the basidia develop over the whole surface of the gill throughout its life. However within this latter group are some species in which, on any one area of hymenium, discrete generations of basidia are developed. Thus in Panaeolus campanulatus the gill is divided into areas of the order of 0.5 sq. mm. which shew a periodic spore discharge with a period somewhat greater than 8 hours. Spore discharge from adjacent areas is out of phase, and thus, since the ripe spores are black, the gill presents a mottled appearance. Sporulation may be considered to take place as a result of a series of irregular waves of development. There are about ten generations of basidia.

In the autumn of 1955, variation was noted in the density of spore deposits on slides exposed for short consecutive periods beneath fructifications of Trametes gibbosa. These observations were made both with

material in the field and with pieces of fruit body in the laboratory.

Further investigation of this species revealed a rhythmical variation in the density of spore deposits obtained on slowly rotating glass discs in defined laboratory conditions. The results of this investigation were published in the Annals of Botany in April 1959 (Gay, Hutchinson and Taggart). This paper is attached as appendix H.*

After a consideration of the possible effects of irregularities in the apparatus and in air currents on the spore patterns (Appendix H pp. 302-303) it was inferred from an analysis of the patterns (pp. 303-304) that the pattern of the deposits had resulted from rhythmical variation in the rate of spore discharge. It was suggested (p. 304, para. 2) that the oblique deposits were the result of 'successive differences in the time of attainment of maximum rates (of sporulation) in adjacent areas of hymenium above the slit'.

It was stated that work was in progress to examine the effect of environmental or internal stimuli in relation to sporulation zones (p. 305) and to examine the distribution of the phenomenon in other Hymenomycetes.

These investigations were begun by considering the following aspects.

A. The selection of convenient material for further work, Trametes gibbosa having been chosen by chance.

B. The investigation of variation in sporulation rate between zones of hymenium in different parts of a single fruit body.

C. Further investigations of the mechanical efficiency of the apparatus.

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* The present author was responsible for the design and construction of the spore collecting apparatus and for the carrying out of much of the experimental work.

D. Examination of the effects of environment.

The first three of these projects had been started when Dr. P. H. Gregory* drew our attention to the possibility of a direct correlation between incubator heater cycling, air currents and spore deposition. Up till this time the investigation of this possibility had been given low priority as no hypothesis involving variation in environment could be produced which could account for an oblique recurrent spore pattern.

However Dr. Gregory's criticism led us to suspend the other work and investigate the possible correlation he suggested. The results shewed that there was a direct correlation between the heater operation and the spore patterns and thus the authors were wrong in interpretating the obliqueness in the spore deposits as evidence against the possibility. However during these investigations other rhythmical spore patterns were obtained which were unrelated to the heater operation. A further section of this thesis gives an account of the investigation of these latter rhythmical patterns. The work on A, B, and C was stopped since the above findings made it irrelevant.

The work and conclusions reached on section D are presented in the main argument of this thesis and the work done on sections A, B, C for its intrinsic interest in appendices G, F and E.

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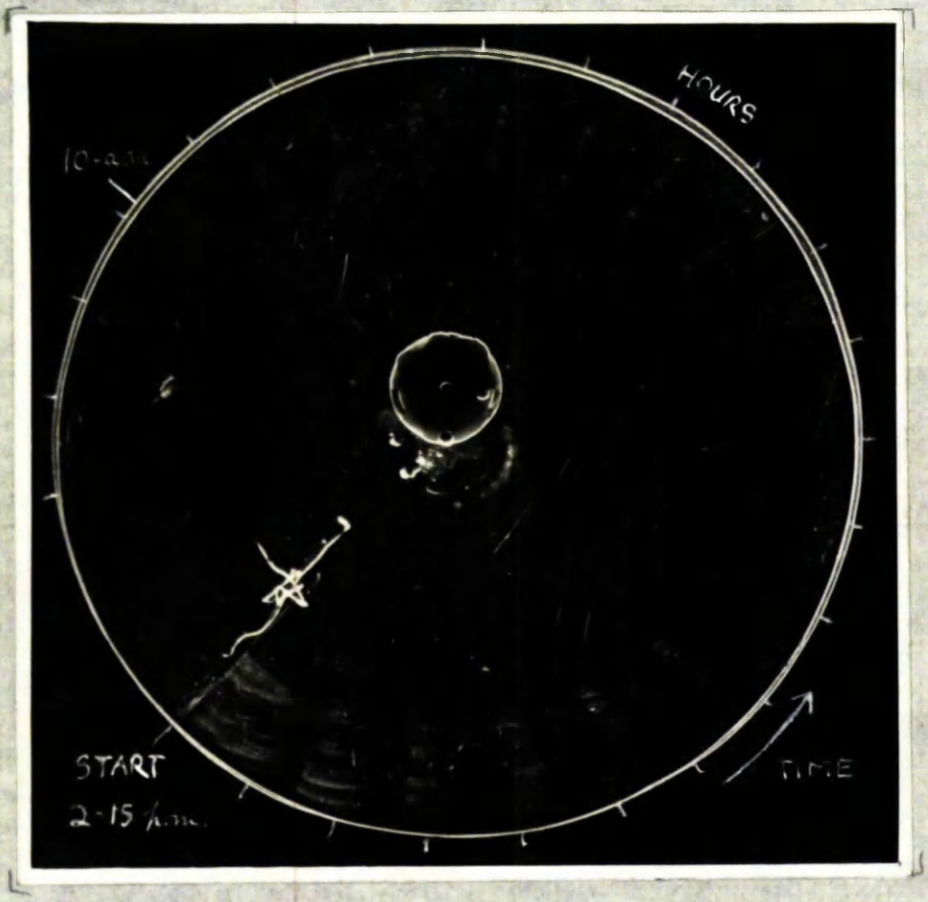
* Dr. P. H. Gregory, Rothamsted - personal communication.

Photograph 1. **Spore Pattern on glass disc - Incubator A.**



Photograph 2. **Record of heater operation during the deposition of the above pattern.**

I. Relation between cyclical Environmental Change in the Incubator and Spore Patterns

This work followed directly on Gregory's suggestion that cyclical air currents might develop through the slit of the collecting apparatus in response to the switching on and off of the incubator heater, and that such currents might control the deposition of spores. However, we bear in mind the alternative that the heater might exercise a direct effect on the sporulation rate of the fruit body.

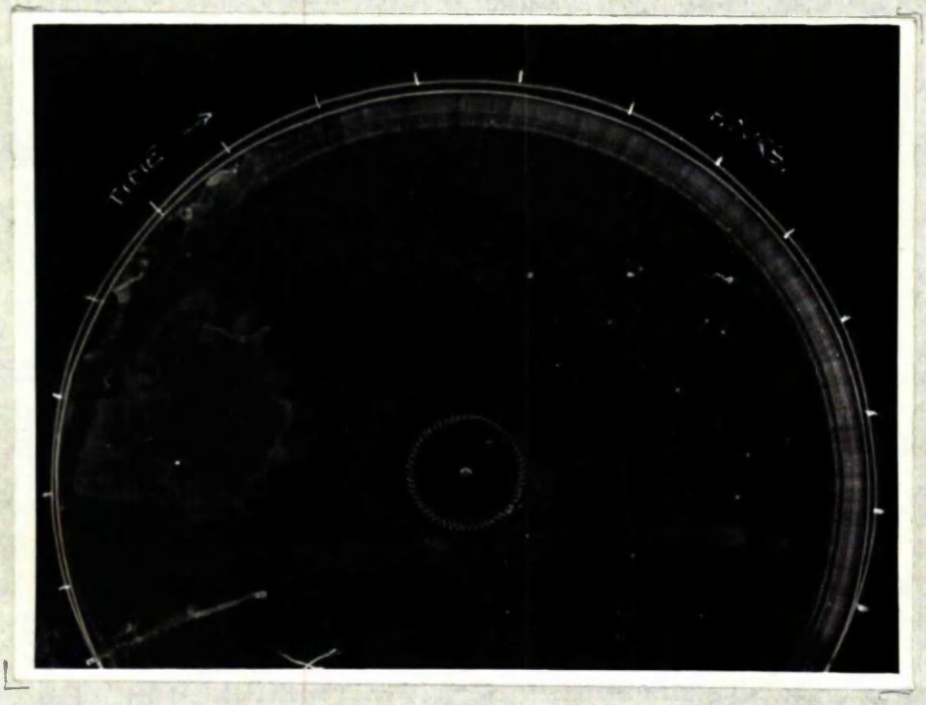
(1) Correlation between Heater Cycling and Spore Patterns

For the investigation of this possible correlation a spore pattern was obtained in the manner, and with the same apparatus, previously described. During the deposition of the spores the operation of the heater was recorded on a rotating drum driven by an electric motor.

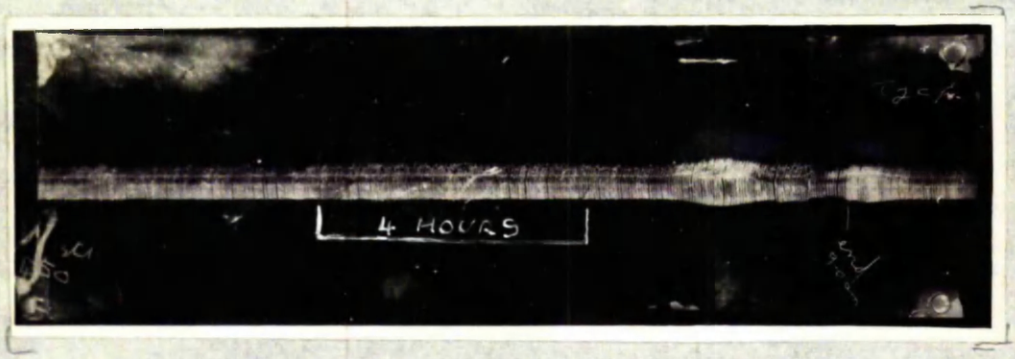
The sources from which the fruit bodies were obtained were recorded but are not thought relevant in the present context. One fruit body grown on a sawdust medium (appendix D) was used along with field material. Four experiments were carried out.

Photograph 1 shews a typical spore print and photograph 2 the corresponding record of heater operation. The number of deposits in the time for which the record was kept is equal to the number of operations of the heater. As the drum on which the record of heater operation was obtained was not driven by a clock mechanism, care must be taken in deriving actual times of heater operation from the record, though the number of operations during the experiment can be precisely determined.

Photograph 3. Spore deposit on glass disc - Incubator B.



Photograph 4. Record of heater operation during the deposition of the above pattern.



The other three experiments confirm this result.

Confirmation of these experiments was sought in another incubator (B) which had a forced circulation of refrigerated cooling water.

One experiment was carried out. The resulting spore print is shewn in photograph 3 and the record of heater operation in photograph 4. The spore pattern shews intervals between maxima of about 15 minutes, but the record of the heater operation shews a three minute period. In another experiment in which a pen recorder was connected to the heater, the period of heater operation was found to be about 2 minutes. No visible spore print was obtained during this experiment. Visual observations on several occasions have confirmed this order of period of heater operation.

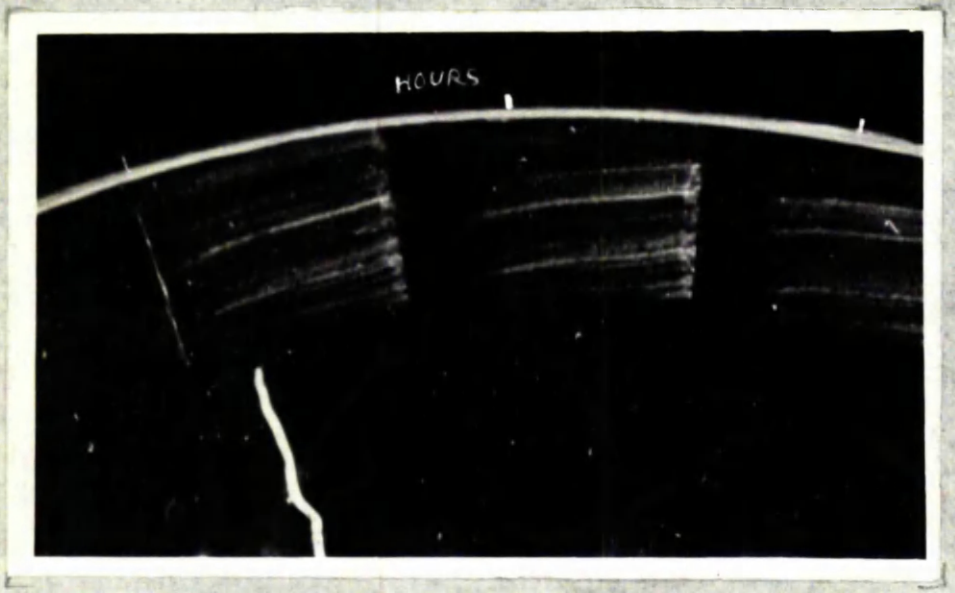
Conclusion. The deposition of spores in this apparatus is clearly correlated with the heater operation in the incubator (A) used in the previous work, but no such correlation exists in incubator B. The spore pattern produced in incubator B is discussed further in II below.

(ii) Nature of Relationship between Spore Patterns and Heater Cycling in Incubator A.

The heater operation may influence either the deposition rate of spores on the collecting surface by a varying air circulation through the slit, on the sporulation rate of the fruit body. The former alternative is first considered by seeking evidence for the draughts that such a process would entail.

If draughts were absent spore fall would be expected to be vertical and thus, were the disc stopped, the spore deposit would be expected to have a sharp radial boundary beneath the slit position. On the other hand it was

Photographs 5, 6 and 7. End portions of spore patterns on removal of discs at specified times relative to the operation of the heater in Incubator A.



Photograph 5

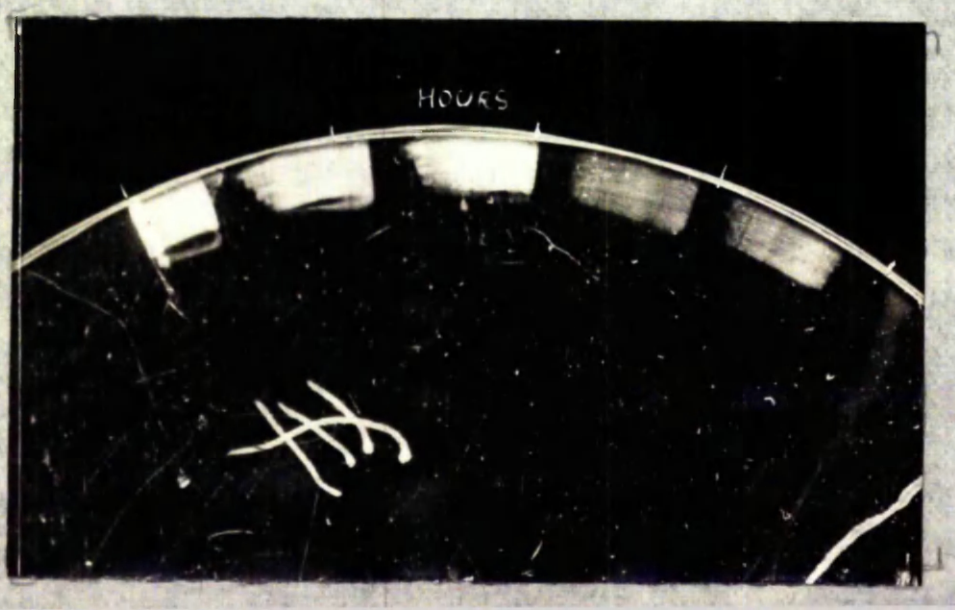
Disc Removed

Heater on + 2 minutes

Photograph 6

Disc Removed

Heater off + 5 minutes



Photograph 7

Disc Removed

Heater off + 10 minutes

realised that such a deposit might be obtained in the presence of a backwards draught, which would not blur the sharp edge between the spore deposit and the unexposed portion of the disc. However, the absence of these draughts would be indicated if the disc were left stationary for some time and the end deposit were found to be a sharp image of the slit. Thus two sets of experiments were carried out. In one set (a) of three experiments the fruit bodies were removed at known times in relation to the heater operation, the part of the disc below the slit being marked at the same time. In the other set (b) of experiments the disc was stopped, and removed some hours earlier.

Set (a). The end portions of the spore patterns are shewn in photographs 5, 6 and 7. Two things are evident; one, that spore deposition is associated with the switching off of the heater, and two, that there is no overlap of the finishing line by the spore deposit. This latter observation indicates that in these experiments there were no draughts which could carry spores on to portions of the disc never exposed beneath the slit, but does not indicate an absence of draught distortion in a backward direction.

Set (b). In the second series of experiments a clutch was fitted to the clock (b) - a newer clock of similar design and reliability to that previously used (clock (a)) - enabling the disc to be put out of gear by releasing a string passed through the thermometer hole of the incubator. This was done three hours before the end of the experiment which was repeated once.

The end portions of the spore patterns are shewn in photographs 8 and 9. It can be seen that the end deposits represent the sum of about 4 and 7 deposits respectively. They are wide and very far from being the simple

Photographs 8 and 9. End Portion of spore deposits obtained in incubator A with disc stationary for three hours before removal.

Photograph 8

Final Deposits

Photograph 9

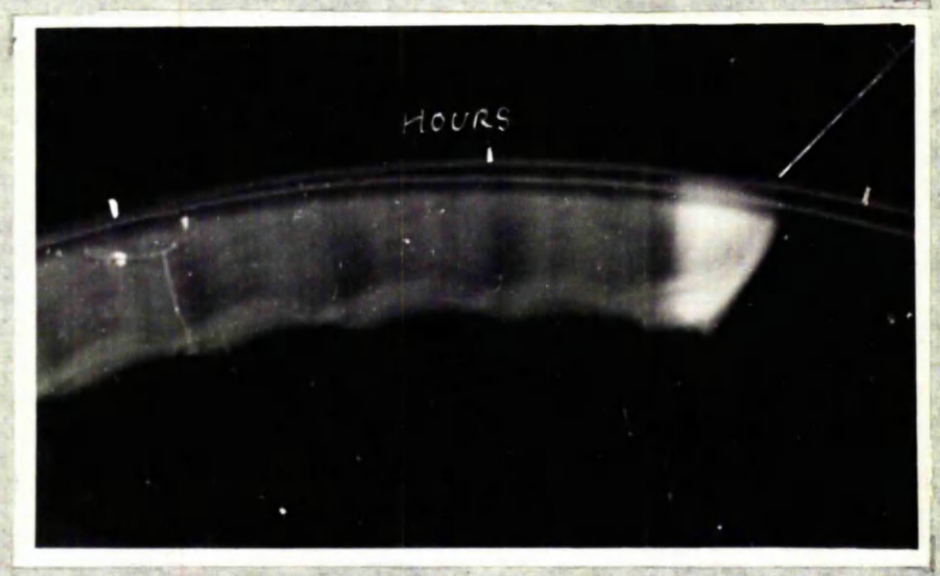


image of the slit to be expected if draughts were absent. It can be seen that these end deposits indicate both forward and backward draughts. Since evidence of forward draughts was not found previously the conditions in the incubator must shew variation from time to time. Such variation is not of any biological interest and has therefore not been investigated further.

From the evidence given in this section it appears that the heater correlated spore pattern is largely determined by air currents through the slit and, thus, we can draw no conclusions about variation in sporulation rate from these patterns.

II. Rhythmical Spore Patterns Unrelated to Heater Cycling

It is noted above that a spore pattern with a 15 minute period was obtained in incubator B while the heater cycled with a three minute period. Other experiments (Appendix F) carried out in incubator B also shewed a 15 minute period. While the heater cycling was not recorded during these latter experiments there is no reason to suppose it to be of a different order to that recorded above. Photograph 7 (the result of an experiment carried out in incubator A - see previous section) shews an indication of a 15 minute variation in density within the major pattern. This has not, however, been noted in other similar experiments - see e.g. photographs 5 and 6.

From these facts it seemed likely that another set of spore patterns could be obtained with the apparatus which would be unrelated to the heater cycling. The following experiments were carried out to confirm this.

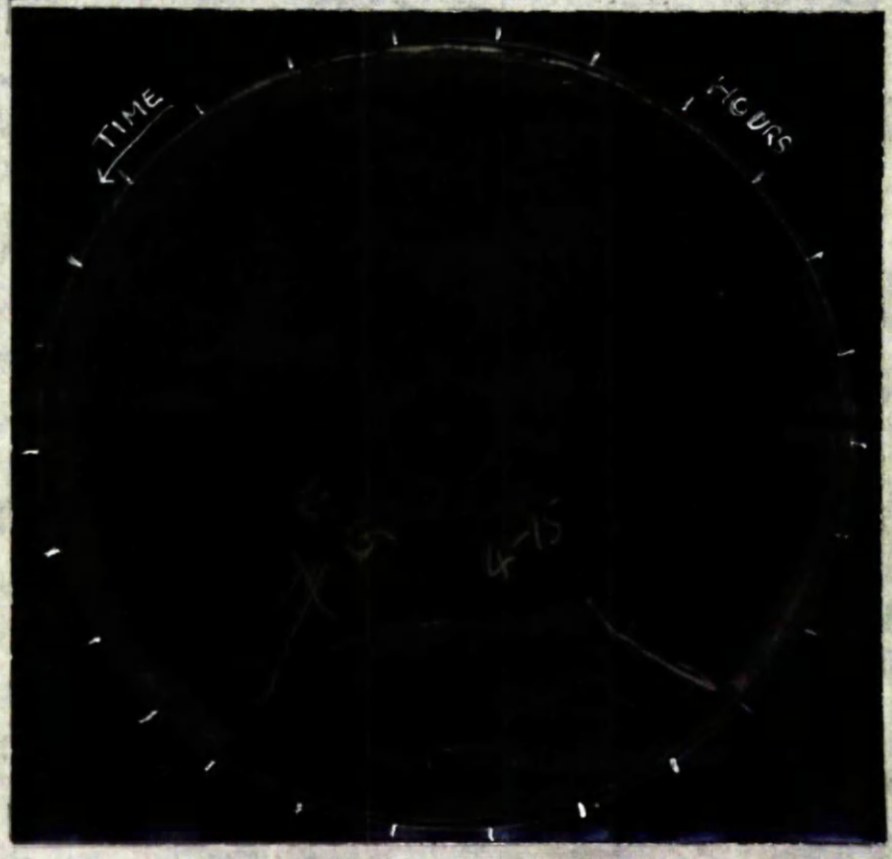
Photographs 10 and 11. Patterns in Incubator A.

Photograph 10.

Heater operating during first part of experiment

Photograph 11.

Heater off all the time



Method.

Experiments were carried out in both incubators, using the original apparatus. Both clocks (a and b) were used to rotate the disc (1 rev. per day). During these experiments some changes were made in the heating system of the building. These resulted in some violent daily temperature fluctuations in the normally steady environment of the laboratory in which the early experiments in incubator A were carried out. This incubator was therefore moved to another room in the same building, with a steadier ambient temperature. Some further experiments were carried out in Trinity College, Dublin, using the same spore collecting apparatus (clock b) and an incubator (C) similar to incubator A.

In incubator A four experiments were carried out in which the heater was working for the first half of the experiment; in three of these a cultured fruit body was used. Three experiments were conducted with the heater off all the time, all with cultured fruit bodies.

In incubator B one experiment was carried out in which the heater was working for the first half of the experiment only. A cultured fruit body was used.

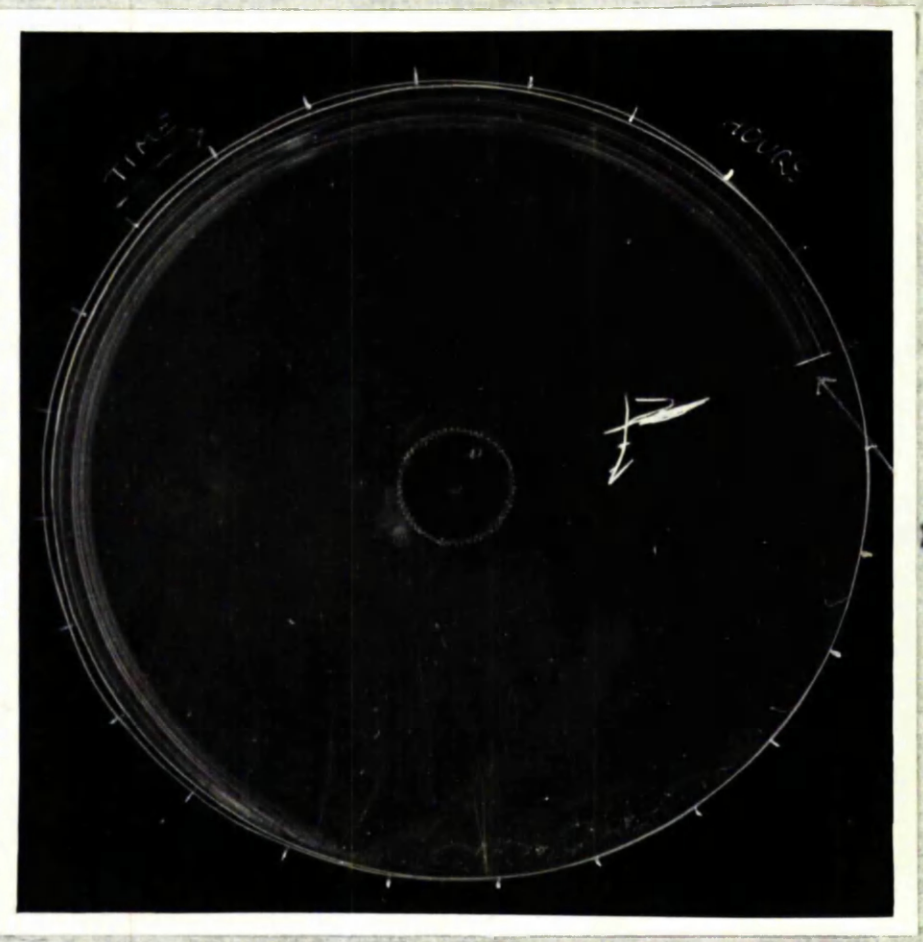
Four experiments were carried out in T.C.D. in which the heater was off all the time. Fresh fruit bodies from two different colonies were used in all experiments.

Results.

Incubator A. Photograph 10 shews the spore print obtained in one of the experiments. The pattern in the part during which the heater was operating shews an interval between maxima of 50 minutes demonstrated to be related to

Photographs 12 and 13. Spore Patterns obtained in incubator A with the heater off shewing an absence of rhythmical variation.

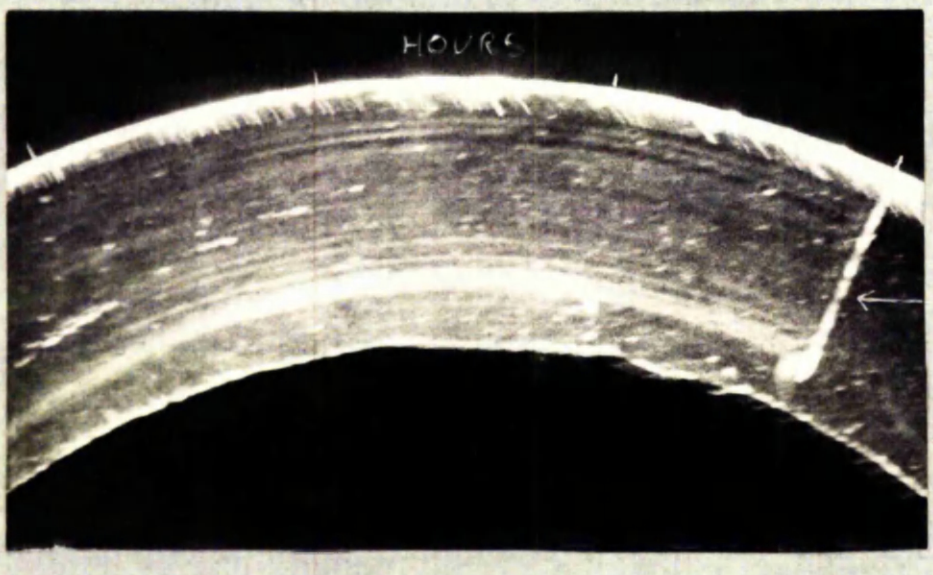
Photograph 12



Photograph 13

Photograph 14. Spore deposit obtained in incubator B with heater off during latter part of experiment.

Photograph 15. Spore Pattern obtained in incubator in Trinity College - Heater switched off.



the heater cycling (see above). The part of the pattern obtained with the heater switched off shews a 15 minute period. Similar though fainter spore prints were obtained in the other three experiments of this kind. Again the periods of the patterns obtained with the heater off were about 15 minutes. All four of these experiments were carried out before the incubator was shifted.

Of the 3 spore prints obtained with the heater off all the time with the cultured fruit bodies, one shewed a 15 minute period (photograph 11), but other two carried out with different fruit bodies shewed no clearly rhythmical pattern (photographs 12 and 13). There is, however, a slight suggestion of a 9 minute rhythm in the latter portion of the pattern in photograph 13.

Incubator B. Photograph 14 shews the spore pattern. An approximately 15 minute interval between maxima can be seen throughout. There is a slight disturbance at the time when the heater and circulator were switched off.

Trinity College Experiments. Photograph 15 shews a portion of one of the spore prints obtained. The spore pattern is very nearly uniform, but slight irregularities prevent us from considering this result as positive evidence for the absence of a recurrent pattern. The other spore prints obtained were similar to that illustrated. In all cases the spore deposit was very slight.

Effects of Draughts on the Deposition of Spores with the Incubator Heater switched off.

In 4 of the above experiments the possibility of draughts affecting the pattern of deposits was examined by the method described in 1(b) above. The images of the slit in such deposits can be seen in photographs 12, 13 and

15. All the edges are sharply defined and it therefore appears that in these cases the spore fall was vertical and not influenced by draughts.

Discussion

It is clear from the evidence given above that the spore patterns with a period of the order of 40 minutes are related to the heater cycling in the incubator. In previous experiments (e.g. Plate 23, appendix H) the thermograph record and the spore pattern shewed some divergence, which was interpreted as indicating an absence of direct correlation. It is now appreciated that this interpretation was wrong; the slight divergences may possibly have been due to the record not having been made of the environment immediately above the incubator heater. The evidence given above for the dominant rôle of air circulation in the deposition of these 40 minute patterns means that no information as to the existence of possible variation in sporulation can be obtained from them. This work also shews that the interpretation of the oblique lines of dense deposit (appendix H) as a reflection of sporulation waves is unjustified. These can, however, be accounted for either by a more or less radial air current of gradually varying intensity or by a more violent puff of air of short duration. It is noted that this latter possibility would involve an extremely great spore production in a very short time. It is significant that the oblique lines tend to be absent in experiments with the heater switched off. Additional evidence that the previous explanation of the oblique lines as evidence of sporulation waves is wrong is given in appendix F which reports experiments in which separate areas of fruit body give the same and synchronous spore patterns.

In incubator A rhythmical spore patterns with a period of about 15 minutes were found in 5 of the 7 spore prints obtained with the heater off, and once superimposed upon the 40 minute now known to be determined by the incubator heater cycling. In the experiment carried out in the incubator B a 15 minute pattern was found both during the period of heater operation and after it had been switched off. In incubator C, 4 experiments were carried out with the heater off and very slight irregularities were noted in the spore deposits obtained, although no clearly defined pattern was visible.

These rhythmical patterns cannot be correlated with any known irregularities in disc rotation (appendix E).

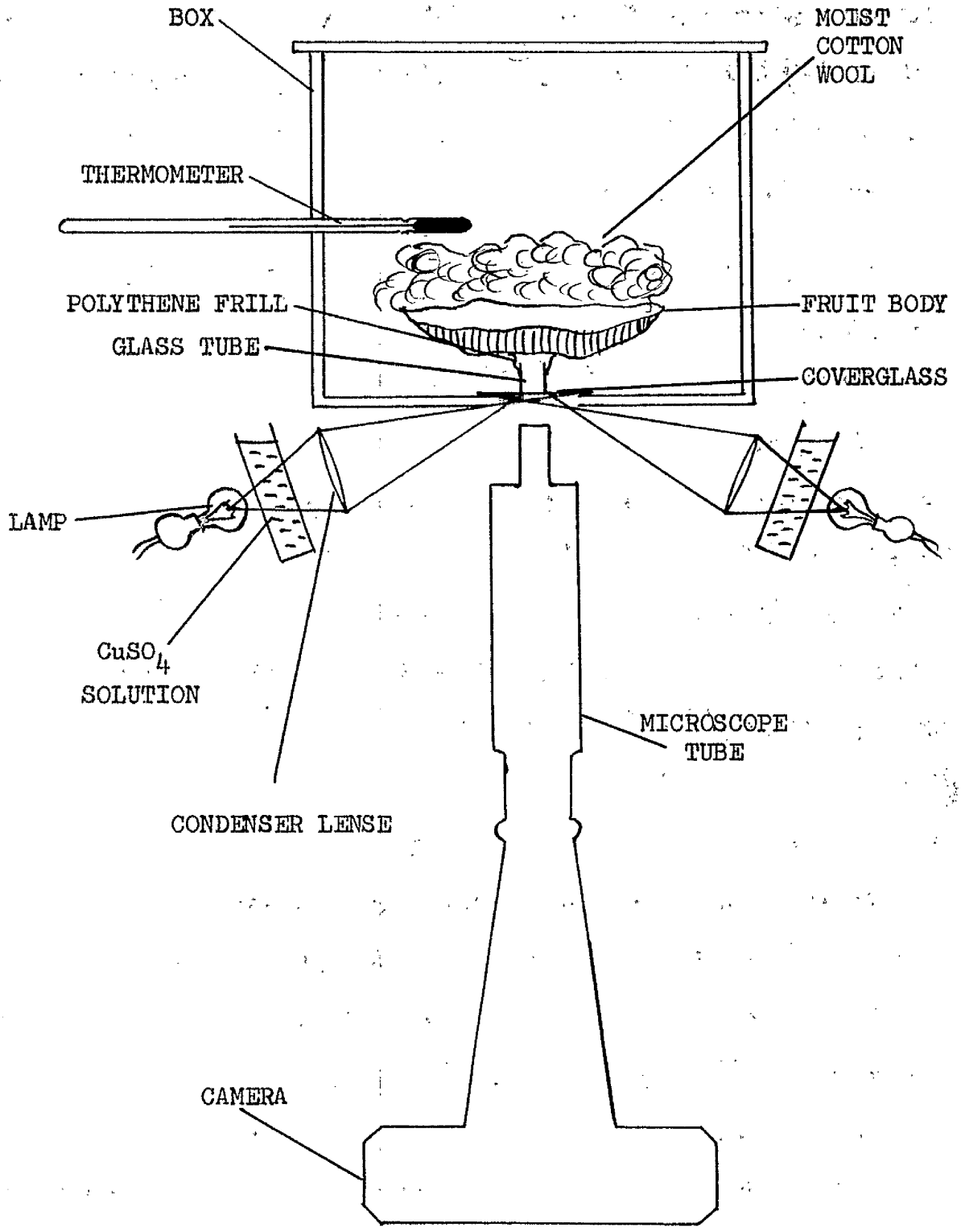
In these conditions the absence of air currents strong enough to disturb vertical spore fall, has been demonstrated in two experiments in incubator A (Photographs 12 and 13), and in two experiments in incubator C (Photograph 15). These experiments were all thought to be precise replicates of the other relevant ones in each incubator, but it is noted that the two experiments, in incubator A for which we have this evidence, were those in which a uniform deposit was found. Also in incubator C the variation in deposit was so slightly marked that no conclusion can be drawn with regard to the presence or absence of a rhythmical pattern.

It is also noted that other small variations in the density of deposit occur in many experiments, some of which may form a rhythmical pattern with two to three minutes between maxima (see e.g. Photograph 14). Both these and the 15 minute patterns are consistently radial with no oblique lines in them.

The present evidence is, therefore, inadequate to support a definite

FIGURE 1.

APPARATUS FOR RECORDING THE DEPOSITION OF SPORES.



x ¼ approx.

explanation as to the cause of the patterns. It is clear, however, that they cannot be correlated with any recorded variations in the apparatus or environment. Besides it is difficult to imagine any rhythmical short-period environmental variant which would be common to a series of insulated boxes, held in a variety of locations.

Investigation as to Uniformity of Sporulation in Trametes gibbosa by Direct Observation

An examination of variation in sporulation rate by observation on the number of falling spores is recorded in appendix H. There were shewn to be significant differences in the numbers of spores falling over consecutive short periods (of the order of 30 minutes). However, the conditions in which these experiments were carried out were not as carefully controlled or as fully investigated as in the rotating disc experiments. These direct observation experiments have therefore been extended using a modified technique.

Apparatus.

Figure 1 shews the apparatus. It consists of a hardboard box about 10" x 8" x 6" with a hole some two inches square in the bottom over which is placed a large coverglass. A microscope (x15 eyepiece, x10 objective) is arranged to look upwards on to the coverglass which is illuminated obliquely from opposite sides, the light first passing through cells filled with $CuSO_4$ solution to filter out heat radiation. A camera is fitted to the microscope. The fruit body is placed in the box above the coverglass and a short section of glass tube is arranged between the pored surfaced and the glass above the objective. The top of this tube has a polythene frill in contact with the fruit body and the base is ground flat and stuck to the

FIGURE 2. RATE OF SPORE DEPOSITION.

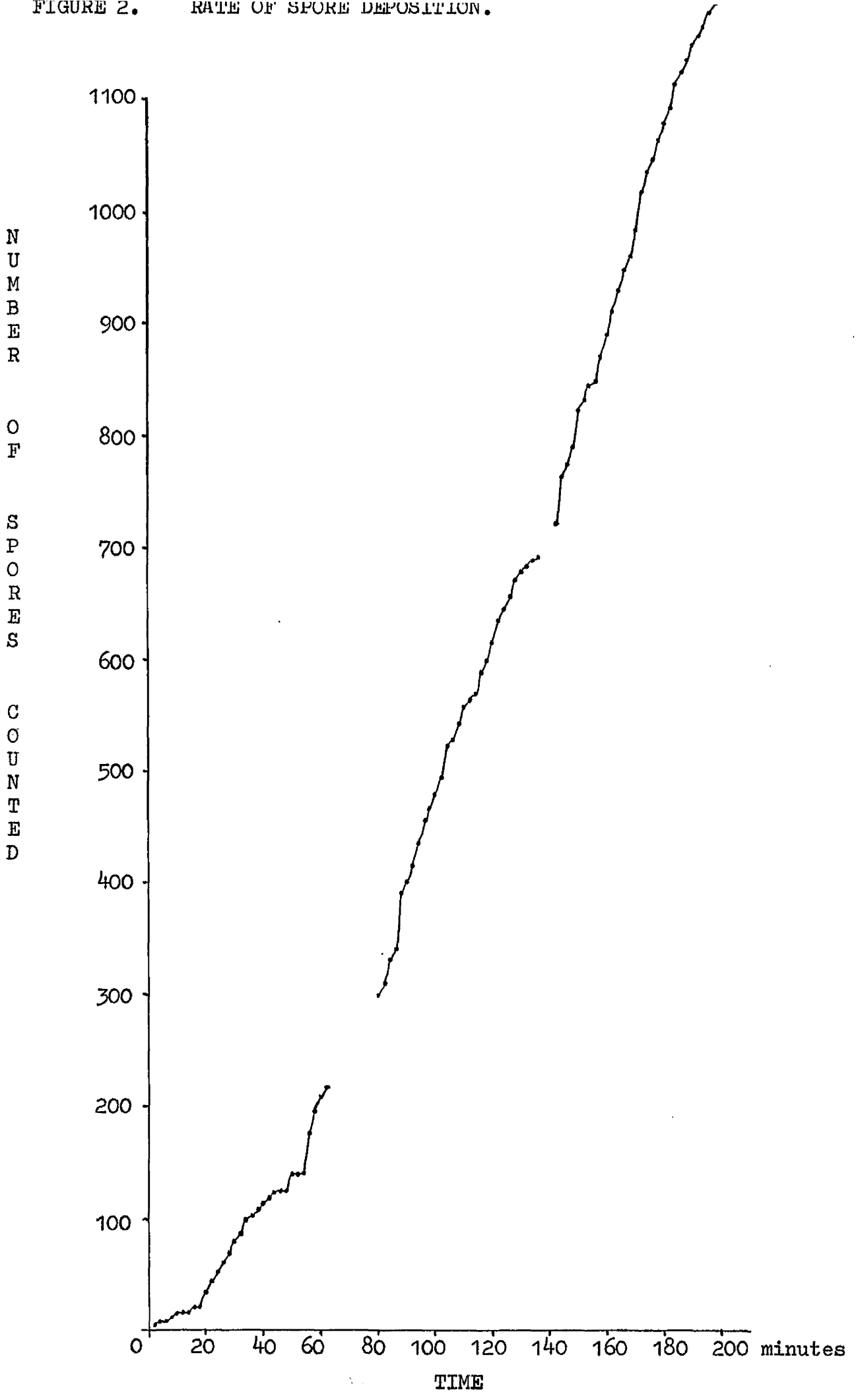
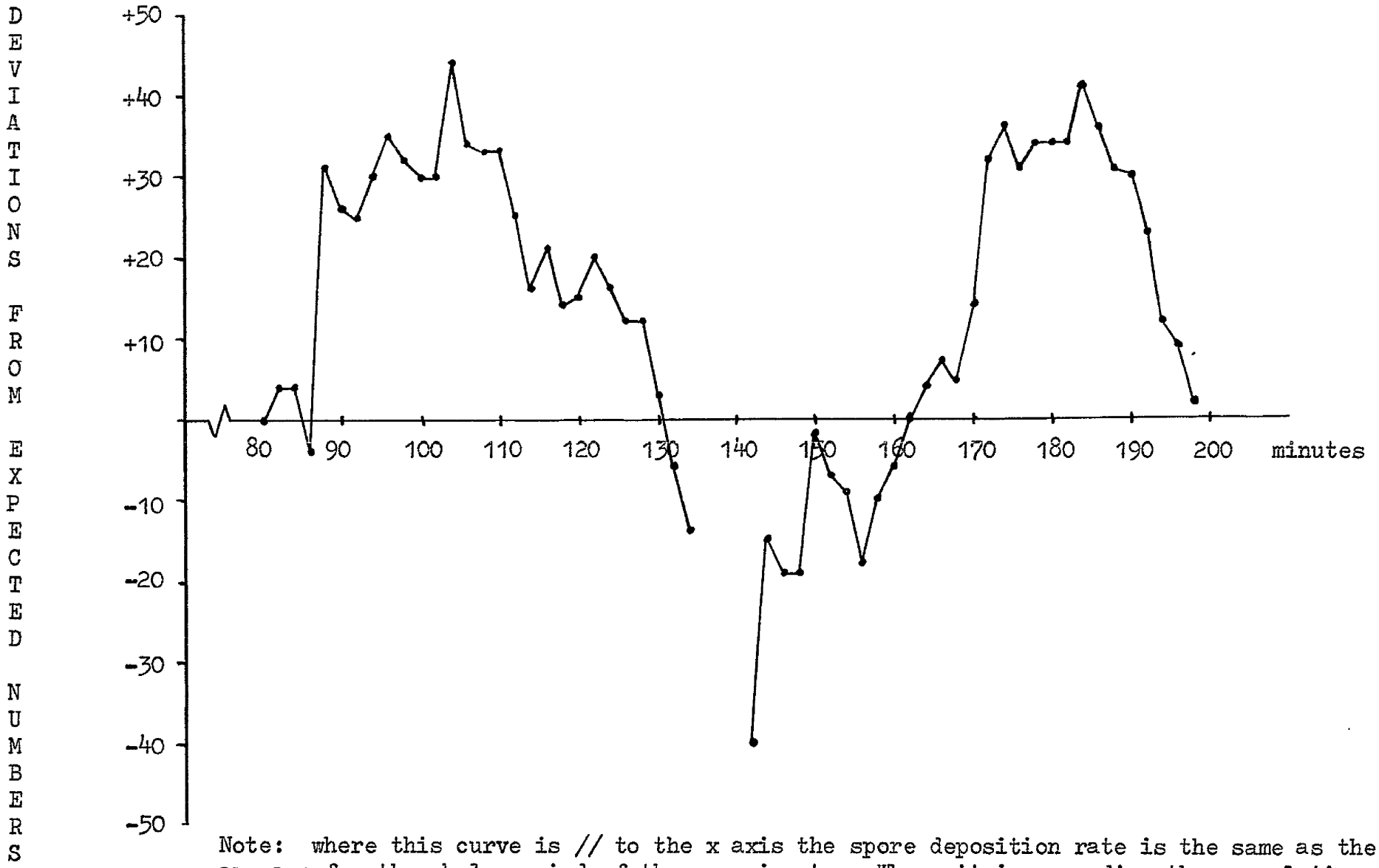


FIGURE 3. Deviations of the numbers of spores counted from the numbers to be expected at given times were the rate of increase uniform.



Note: where this curve is // to the x axis the spore deposition rate is the same as the average for the whole period of the experiment. Where it is ascending the sporulation rate is greater and where it is descending the spore deposition rate is less than the average. The initial period of the experiment is not shewn on this figure.

coverglass with sellotape. Wet cotton wool is placed over the fruit body and a thermometer is set into the box.

Method.

The experiment was carried out in a basement room with the heating turned off to ensure as little temperature variation during the experiment as possible.

The fruit body used in this experiment came from a sycamore stump in Angus. It was placed above the coverglass and photographs were taken of the accumulating spore deposit at two minute intervals. The illumination was only switched on for a few seconds for each exposure. The temperature was recorded about every 15 minutes and the experiment was continued for 196 minutes.

The fruit body and apparatus were left undisturbed for two days after the end of the experiment and then the spore deposit on the coverglass was photographed at a lower magnification.

The first photograph of the spore deposit was projected on a screen and the position of each spore marked. Successive photographs were projected on to the same screen and in each the additional spores were counted as they were marked off.

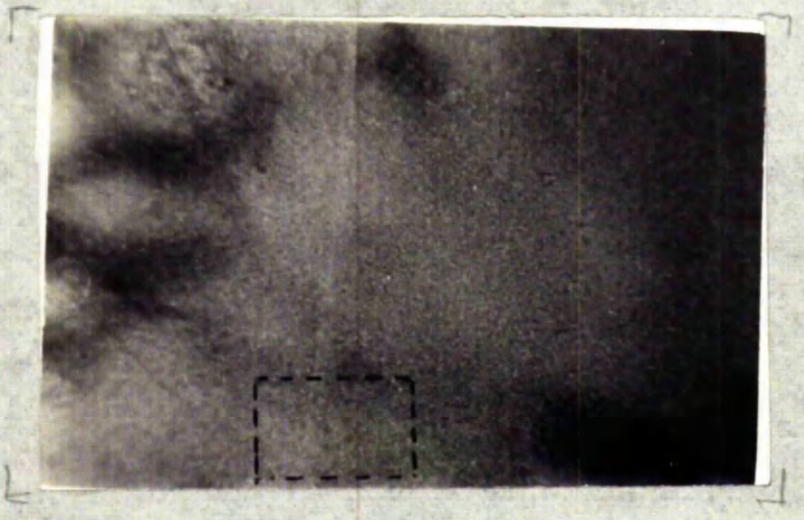
Results.

Figure 2 shews the increase in the number of spores against time and figure 3 the deviations of the number of spores counted at a given time from the number to be expected at that time were the rate of increase uniform. The gaps in these two graphs result from the time taken to change the film cassettes.

Photograph 16.

Spore deposit on coverglass after 2 days.

Note the dark areas indicative of the positions of the dissepiments.



Approximately
x 3

Area on which counts were made.

Photograph 16 shews the deposit on the coverglass after two days sporulation. The portion on which the microscope was focussed is marked. A more or less distinct outline of the pore walls can be seen. This photograph thus indicates that spore fall must have been relatively undisturbed by draughts, and thus the rate of deposition may be expected to reflect the sporulation rate.

The temperature during the experiment varied from 17.0 - 17.3°C.

Discussion

Though there are peaks in the graph at approximately 100 and 200 minutes from the start of the experiment, the deviations in figure 3 are very small and it is not possible to analyse their significance from this single experiment. They give, therefore, no evidence of any major variation in this case which can be correlated with the variation found in the rotating disc experiments (see II above). This discussion is expanded therefore to consider the possibility of modification of the technique to investigate the possible existence of real variation in spore deposition rate which cannot be reckoned to have been demonstrated in the above experiment.

With the experiment in its present form certain difficulties arose in the counting of the spores. Optical abberations resulting from the mode of illumination tended to make the separation of spores lying close together difficult. Some errors in the counting may have arisen for this reason when a fresh spore was deposited very close to one already on the coverglass. The chances of this having happened will obviously have increased as the deposit thickened. However, in this particular experiment it is thought that the

errors, resulting from the counting of two or more spores as one, are trivial during at least the first 140 minutes or so. In the latter part of the experiment it cannot be said how much error in the counting has resulted from the confusion of spores, but the continuing steady trend of the curve suggests that it may not have been very great. A small percentage of spores, perhaps 2-3%, were found to turn over on the coverglass some time after deposition. It is thought that the errors arising from this were small, although they are liable to have been greater in the latter part of the experiment where the greater spore density made confusion of spores more likely.

When these difficulties are considered it is obvious that an experiment must be of limited duration. The more vigorous the sporulation rate the shorter must be the duration. Yet it is necessary to count larger numbers of spores if any conclusions are to be drawn from the counts. To increase the number of spores counted by photographing at a lower magnification would, however, make spore separation even more difficult.

To overcome some of these difficulties it might, however, be possible to develop an apparatus along the lines of that used by Hopper and Laby (1941) for observation of falling oil drops. In this the falling particles pass right through the apparatus and thus do not confuse the later observations. Observations are made with a horizontal microscope and a specially designed system of dark ground illumination. This method has not been pursued owing to the difficulty of constructing such apparatus.

Summary of Conclusions

In Part I a value of - (1.35 ± 0.12) x 10^{-8} e.s.u. has been found as the charge on the mean dry rot spore. This charge is of the order to be expected if the charge were acquired by simple physical separation of the spore from the sterigma. No special mechanism is therefore suggested for the acquisition of this charge. Charges of this order of magnitude cannot have any significant effect on the liberation of spores from narrow pores.

In Part II it has been demonstrated that spore liberation on displacing the pores of Polyporus betulinus is diminished to an extent predicted on the assumption that it is determined only by the initial violent projection of the spore and the gravitational attraction alone. Thus it is concluded that no other significant forces are operative in the process.

In Part III previously described spore deposition rhythms in Trametes gibbosa have been shewn to result from the operation of an incubator heater and cannot therefore be interpreted as sporulation rhythms.

In conditions where the incubator heater was not operating, spore deposition rhythms with a period of about 15 minutes have been recorded in some but not all of the experiments. They cannot be related to any known environmental variable. Further interpretation of such rhythmical spore deposition would require additional experimental work, which it has not proved possible to carry out under present circumstances.

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Table IA

Previous Estimates of Daily Spore Production for the Fruit Bodies of Various Species

Species	Daily Spore Production x 10^6	Authority
Fomes fomentarius	55,000 to 60,000	Buchwald 1937
" "	378	Meyer 1936
Ganoderma applanatum	30,000	White 1919
Serpula lacrimans	10,000*	Ferdinandsen & Buchwald 1937
Polyporus squamosus	3,571	Buller 1922
Agaricus campestris	2,666	Buller 1922
Coprinus comatus	2,620	Buller 1922
Coprinus stercqulinus	100**	Buller 1922
Daedalea confrugosa	97	Buller 1922

* Derived from Ferdinandsen and Buchwald's figure assuming a fruit body life of a hundred days ('several months' - F. & B.).

** Spore production for life of fruit body (8 hours).

Appendix A

Numbers of Spores Produced by Various Species

It has been known for a long time that vast numbers of spores are produced by fruit bodies of the Agaricales. The actual rate of spore production for some common species was first recorded by Buller (1909). More recently other observers have added to the list of species investigated (Table IA). In the course of the work described in this thesis, the orders of magnitude of spore production for some further species have been determined. The results have been expressed in Table IB as the number of spores produced per fruit body per 24 hours. While a value for the rate of spore production per unit area of hymenium per unit time might be of greater interest the results have been expressed in the above form because of a lack of information as to the distribution and extent of hymenium in some of the species. The investigation of this is thought to be without the scope of the present thesis. The expression of the results in their present form also enables direct comparison to be made with previously determined values.

The methods by which the values have been derived for each species are given below.

Polyporus betulinus

The data given in Part II is used. A typical fruit body is taken as having a surface area of 10^4 sq. m.m. (see e.g. the floras cited in Table I, Part II) and the pore surface density as being 10 pores / sq. m.m. (Table I, Part II). That is, the fruit body is assumed to contain 10^5 pores. For

Table IB

Estimates of Daily Spore Production for the Fruit Bodies of Various Species

Species	Daily Spore Production x 10^6		Approximate Temperature
Polyporus betulinus	50,000		20°C
" "	10,000		14°C
" "	7,500		6°C
" "	150		3°C
Serpula lacrimans	10,000	from c. ½ sq.ft. of fruit body	20°C
" "	10,000		20°C
Boletus edulis	600		8°C
Trametes gibbosa	1,000		17°C

fruit bodies in their normal orientation, the following orders of spore production were found.

Spores/pore/minute	Approximate temperature
300	20°c
70	14°c
50	6°c
1	3°c

Calculation from this data gives daily rates of spore production of 50,000, 10,000, 7,500 and 150 million respectively.

Serpula lacrimans

The values for spore production in this species have been calculated from the data obtained in the estimation of spore mass (Part I). In this work the spores from about 3 sq. feet of fruit bodies were collected over periods of about 2 and 2¼ days. Though the totals of the collected spores were not weighed, nearly all the spores were used in weighing the several batches and thus the total weights are approximately known. These total weights are taken as 0.12 and 0.16 gms. This gives in each case a daily spore production of 0.06 gms. The estimates of spore mass indicate that this is equivalent to about 10,000 million spores per day.

Trametes gibbosa

In the experiment described in the final section of Part III a spore deposition rate of about 400 spores per hour was obtained on an area of about 0.1 sq. m.m. As the spore deposit round about this area was fairly blurred, the outline of the pore orifices being indistinct in the spore print, the value above was taken to represent a mean rate of spore liberation

from 0.1 sq. m.m. of fruit body surface. As fruit bodies of this species are of the order of 10 sq. m.m. (see any convenient flora) there is an estimated daily production of about 1,000 million spores.

Boletus edulis

The Boletus value was calculated in the same way as the Polyporus betulinus ones. The number of pores in the fruit body was estimated from a photograph of the fructification used in the experiment mentioned in Part II. A value of 600 million spore / day was obtained.

Appendix B

Table I

Predominant Sign of Charges in Spore Populations

Species	Predominant Sign	Number of fruit bodies on which observations were made
Polyporus squamosus	+ ve	one
Trametes gibbosa	+ ve	four
Polystictus versicolor	Symmetrically charged	one
Serpula lacrimans	- ve	six

Appendix B

The predominant sign of the charges on the spore populations of various species

In the course of the work which lead to the estimation of the electrostatic charges on dry rot spores (Part I), a number of preliminary experiments were carried out with other species, in which only the sign of the charge on the majority of spores was determined.

Some of these experiments were made with a simpler apparatus than that described in Part I. It was closely similar to that used by Gregory (1957) except that the charged plates were covered by thin slips of glass that could be removed for inspection. A potential difference of the order of 100 volts was used across the plates which were separated by 1.2 cms. For the observations on Polystictus versicolor and Serpula lacrimans the apparatus described in Part I was used. All the experiments were made in the laboratory.

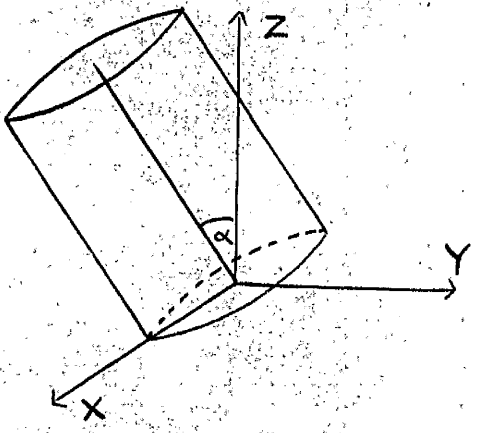
When the slips of glass were examined after some hours a considerable difference between the deposits on the positive and negative sides was usually noted.

The results are shewn in Table I. The print on the base of the apparatus obtained in the experiment with Polystictus versicolor is unusual in that it is symmetrically distributed about the centre of the base plate, indicating a symmetrical distribution of charges in the population about zero.

Appendix C.

We are grateful to Dr. R. P. Gillespie of the Mathematics Department of the University for contributing this appendix.

Derivation of an expression defining the area of the inner curved surface of a cylinder with its long axis displaced from the perpendicular, from which particles could fall vertically through the orifice.



Let the axis of the cylinder be

$$\frac{x}{\sin\alpha} = \frac{y}{0} = \frac{z}{\cos\alpha}$$

and the radius of cross section r

The equation of the cylinder is

$$r^2 = xy^2\cos^2\alpha + (z\sin\alpha - x\cos\alpha)^2 + y^2\sin^2\alpha$$

i.e. $$r^2 = y^2 + (z\sin\alpha - x\cos\alpha)^2$$

Let the flat base of the cylinder be

$$z = -\tan\alpha\left(x - \frac{r}{\cos\alpha}\right)$$

Projection of the intersection of base of cylinder on z = 0 is

$$r^2 = y^2 + \left[-\frac{\sin^2\alpha}{\cos\alpha}x - x\cos\alpha + \frac{r\sin^2\alpha}{\cos^2\alpha}\right]^2$$

i.e. $$r^2 = y^2 + \left(\frac{x}{\cos\alpha} - r\tan^2\alpha\right)^2$$

i.e. $$r^2\cos^2\alpha = y^2\cos^2\alpha + \left(x - r\frac{\sin^2\alpha}{\cos\alpha}\right)^2$$

On upper part of cylinder

$$z \sin\alpha = x \cos\alpha + \sqrt{r^2 - y^2}$$

$$\therefore p = \cot\alpha \qquad q = \frac{1}{\sin\alpha}\left(\frac{-y}{\sqrt{r^2 - y^2}}\right)$$

$$\sqrt{1 + p^2 + q^2} = \sqrt{1 + \frac{\cos^2\alpha}{\sin^2\alpha} + \frac{y^2}{(r^2 - y^2)\sin^2\alpha}}$$

$$= \frac{1}{\sin\alpha}\sqrt{1 + \frac{y^2}{r^2 - y^2}} = \frac{r}{\sin\alpha\sqrt{r^2 - y^2}}$$

Required surface is $\iint \frac{r}{\sin\alpha} \frac{dx\,dy}{\sqrt{r^2 - y^2}}$ over the ellipse $\frac{x^2}{\cos^2\alpha} + \frac{y^2}{1} = r$

Let $x = \xi \cos\alpha$

$y = \eta$

Surface $= \frac{r\cos\alpha}{\sin\alpha} \iint \frac{d\xi\,d\eta}{r^2 - \eta^2}$ over the circle $\xi^2 + \eta^2 = r^2$

$$= \frac{4r\cos\alpha}{\sin\alpha} \int_0^r d\eta = \frac{4r^2\cos\alpha}{\sin\alpha} \qquad \text{I}$$

The top of the cylinder, assuming that its height is h, is in the plane

$$z - h\cos\alpha = -\tan\alpha\left(x - \frac{r}{\cos\alpha} - h\sin\alpha\right)$$

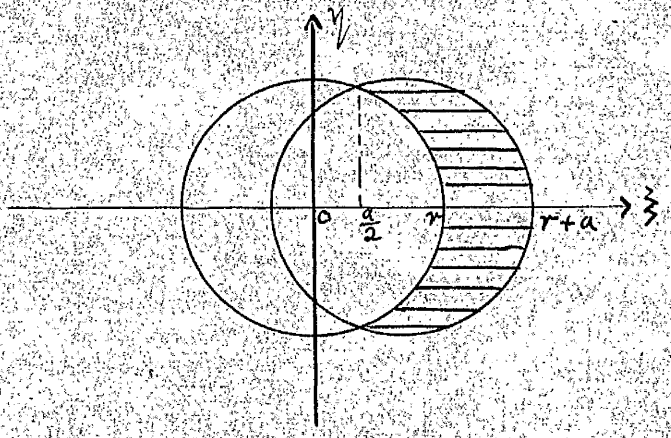
i.e. $z = x(-\tan\alpha) + \frac{h}{\cos\alpha} + r\frac{\tan\alpha}{\cos\alpha}$

Its projection is

$$r^2 = y^2 + \left(\frac{x}{\cos\alpha} - r\tan^2\alpha - h\tan\alpha\right)^2$$

Hence we must integrate now over the shaded area between the circles

$\xi^2 + y^2 = r^2$ and $(\xi - a)^2 + y^2 = r$ where $a = h \tan \alpha$



This is in two parts.

$$(i)\quad 2\int_0^{\sqrt{r^2 - \frac{a^2}{4}}} d\eta \int_{\sqrt{r^2-\eta^2}}^{a+\sqrt{r^2-\eta^2}} \frac{d\xi}{\sqrt{r^2-\eta^2}} = 2a \int_0^{\sqrt{r^2 - \frac{a^2}{4}}} \frac{d\eta}{\sqrt{r^2-\eta^2}} = 2a \sin^{-1} \frac{\sqrt{r^2 - \frac{a^2}{4}}}{r}$$

$$(ii)\quad 2\int_{\sqrt{r^2 - \frac{a^2}{4}}}^{r} d\eta \int_{a-\sqrt{r^2-\eta^2}}^{a+\sqrt{r^2-\eta^2}} \frac{d\xi}{\sqrt{r^2-\eta^2}} = 4\left[r - \sqrt{r^2 - \frac{a^2}{4}} \right]$$

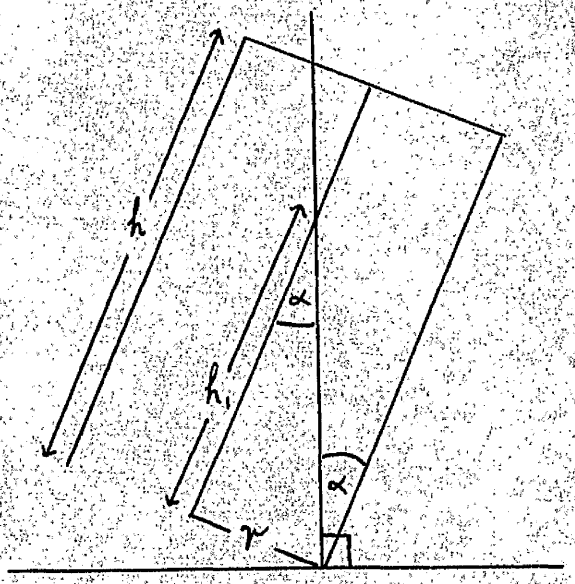
Required surface is $r \cot \alpha \left[2h \tan \alpha \sin^{-1} \frac{\sqrt{r^2 - \frac{h^2 \tan^2 \alpha}{4}}}{r} + 4\left\{ r - \sqrt{r^2 - \frac{h^2 \tan^2 \alpha}{4}} \right\} \right]$

II

Formula I is to be used when $\tan \alpha \geqslant \frac{2r}{h}$

Formula II is to be used when $\tan \alpha \leqslant \frac{2r}{h}$

Derivation of an expression defining the area of the inner curved surface of a cylinder with its long axis displaced from the perpendicular, from which particles, discharged normal to the surface into the centre, could pass, by subsequent vertical fall, through the orifice.



$h_1 = r \cot \alpha$

Case$_1$ when $h > h_1$

i.e. when $h > r \cot$

Particles will be able to pass through the orifice from the surface of the cylinder of height (h_1) and radius (r).

Required surface $= 2 \pi r h_1 = 2 \pi r^2 \cot \alpha$

Case$_2$ where $h < h_1$ i.e. when $h < r \cot \alpha$

Here particles can pass through the orifice from the whole surface area of the cylinder.

i.e. $2 \pi r h$.

Appendix D

Culture of Trametes gibbosa fruit bodies

Introduction

Because of the comparative rarity of Trametes gibbosa in the field it was found convenient to culture fruit bodies for use in some of the experimental work described in Part III.

Isolations

The initial isolations were made on 2% malt agar from internal fruit body tissue, and in a few cases from fragments of infected wood. The cultures were incubated at 20°C and stock cultures kept in the refrigerator at about 6°C.

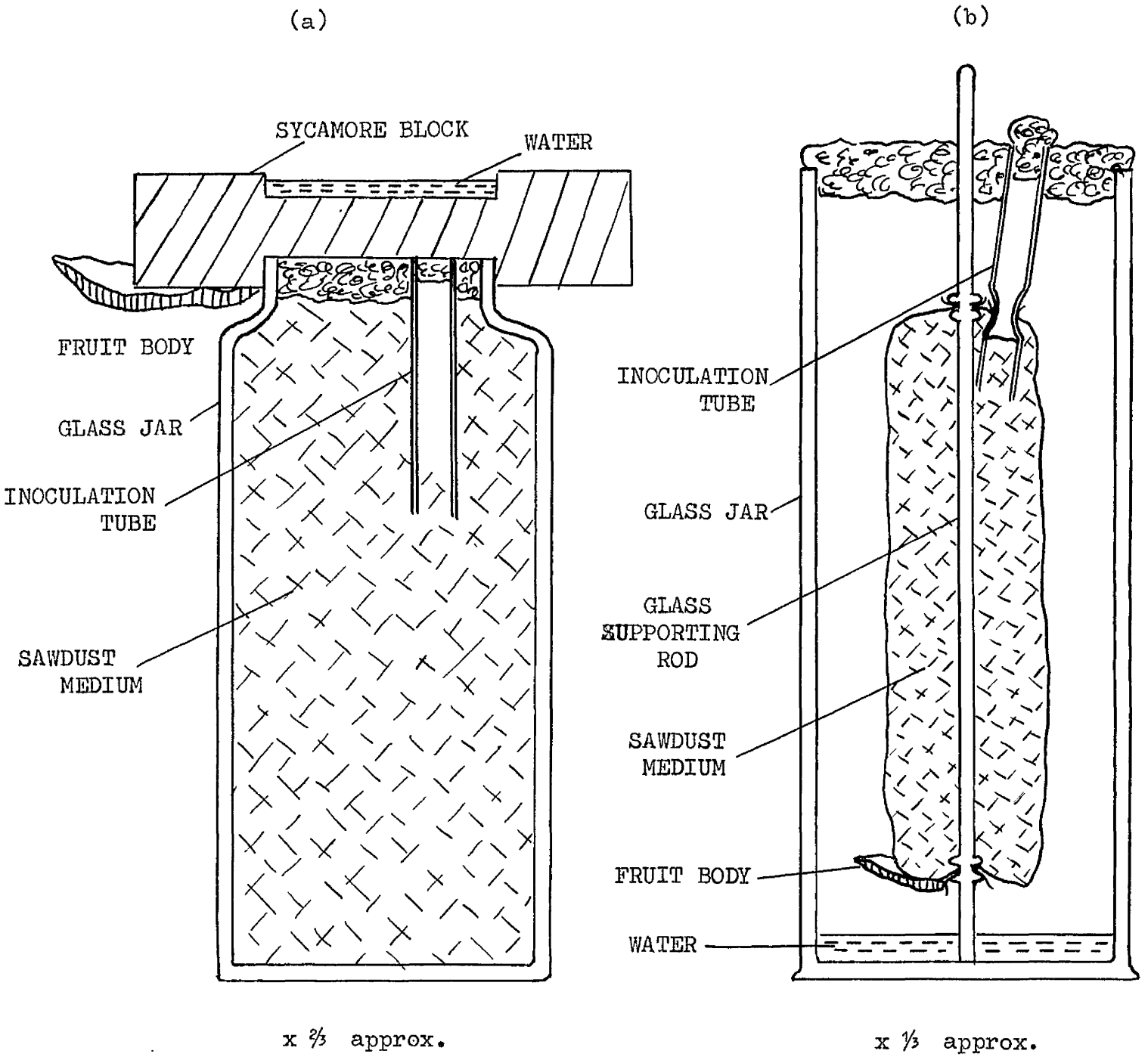
Medium for Fruit Body Culture

A sawdust medium was used. To every 100 parts of sycamore sawdust 5 parts of the following accelerator were added.

	parts by weight
Maize meal	50
Bone meal	30
Potato starch	17
Sucrose	2

This is essentially Badcock's (1941) medium with the omission of wood ash. The moisture content of the sawdust was brought to about 170%.

Figure 1. CULTURE VESSELS IN MEDIAN SECTION



Method

The above medium was used in two ways.

(1) Glass jar - wood block cultures

The method of Tamblyn and Da Costa (1958) was followed.

Suitable jars were filled with medium and stoppered with a cotton wool bung. A small tube passed through each bung into the medium and through these the jars were inoculated (figure 1(a)). The jars were sterilised, and inoculated with Trametes mycelium from the 2% malt agar cultures. They were then placed in a 20°C incubator.

After 3 to 4 weeks the mycelium approached the medium round the cotton wool bungs. At this stage the bungs were moistened and the jars stood in a dish of water and covered with polythene bags to maintain a high humidity.

When the mycelium was growing vigorously out of the necks of the jars, suitable blocks of soaked sycamore wood were fitted over the necks. These blocks had circular holes about 2" in diameter and a ½" deep cut in their upper surfaces. These holes were filled with water at daily intervals during the subsequent inoculation. At this stage the cultures were transferred to small (c. 2' x 2' x 2') polythene sided chambers in a controlled environment room kept at 20°C. Free water was kept in the chambers and lighting from fluorescent tubes was given for 16 hours a day. The amount of light reaching the cultures varied, as the room was used for various other purposes.

After about three weeks mycelium appeared on the surface of the blocks, especially on the top, the sides cut across the grain and round the neck of the jar. In about 50% of cases, this superficial mycelium

resolved itself into fruit body initials and small fruit bodies, fairly characteristic of the species developed and persisted for some months. These fructifications, though small (the largest being less than 2" across), sporulated quite freely. Frequently the pored area extended over the undersurface of the block. Abnormal fructifications with pored upper surfaces occasionally developed on the tops of the blocks.

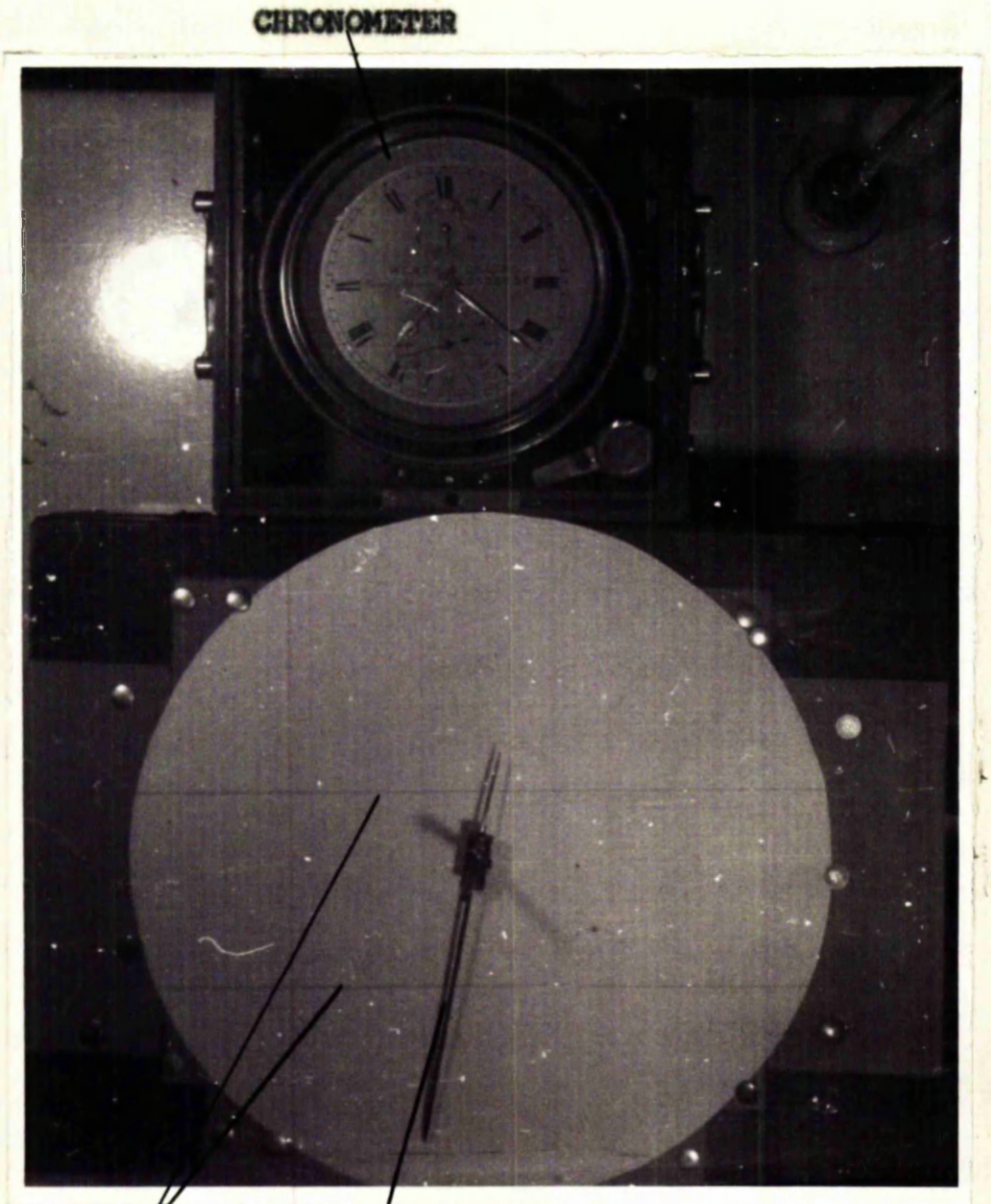
(ii) Glass fibre sac cultures

The sawdust medium described above was again used, but this time it was enclosed in a cylindrical bag about 10" x 3" made of an open weave glass fibre fabric and supported on a glass rod. A tube projected from the top of the bag out of the neck of a tall cylindrical glass jar in which the medium was sterilised (figure 1(b)). Through this tube the sac was inoculated. Water was put in the bottom of the jar to maintain the humidity. These cultures were put with the others in the controlled environment room.

When after about 10 weeks the mycelium was growing freely over the surface of the sac the bung was loosened and additional water poured over the culture at daily intervals. At this point in one culture a fruit body began to develop at the base of the sac. This persisted and sporulated for some months becoming about 2" in diameter. The other three cultures failed to develop fruit bodies.

Although this latter technique was a doubtful success it is felt that it offers the possibility of growing cultures in conditions of better aeration than those found within a glass jar.

Photograph 1. **Apparatus recording regularity of disc rotation.**



x x

Appendix E

Further Examination of Regularity of Disc Rotation

The observations made visually and mechanically on the rotation of the collecting disc (Gay, Hutchinson and Taggart 1959 - see appendix H) were carried out over relatively short periods and only involved one of the clocks used in the later work. It was considered desirable to extend these tests to cover longer periods and both clock mechanisms.

Method

The spore collecting apparatus and a fruit body of *Trametes gibbosa* were set up in the incubator as previously described. The axle on which the disc was mounted was extended through the thermometer hole of the incubator. To the top of the axle a pointer (photograph 1) was attached so that it rotated immediately above a chart fixed on top of the incubator. Beside this chart was an N.P.L. tested chronometer. An automatic camera was arranged such that the pointer, chart and chronometer were photographed at about 15 minute intervals in the test of the one clock and at 12 minute intervals in the test of the other.

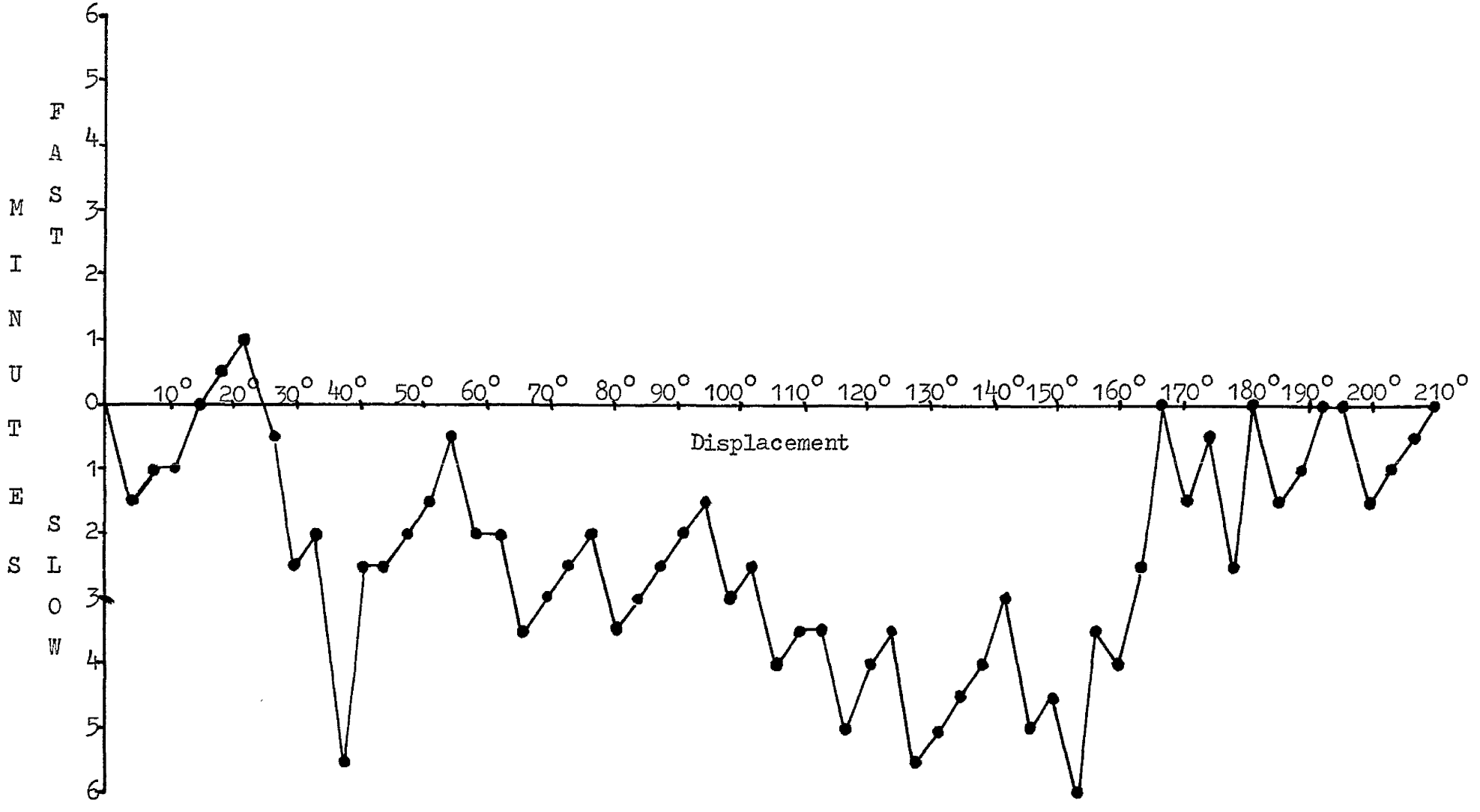
The photographs were projected, the time read from the chronometer and the positions of the pointer and the two parallel lines on the chart (photograph 1) recorded. Two lines were used on the chart to ensure greater accuracy in measuring the angle of intersection with the pointer.

Each of the clock mechanisms (a & b) was tested once in this way.

Figures 1 and 2 shew the extent in minutes by which the clock was ahead

Figure 1. Clock (a).

Deviations from times at which particular displacements might be expected were the rotation rate uniform.



Photograph 2.

Spore pattern obtained with clock (a).

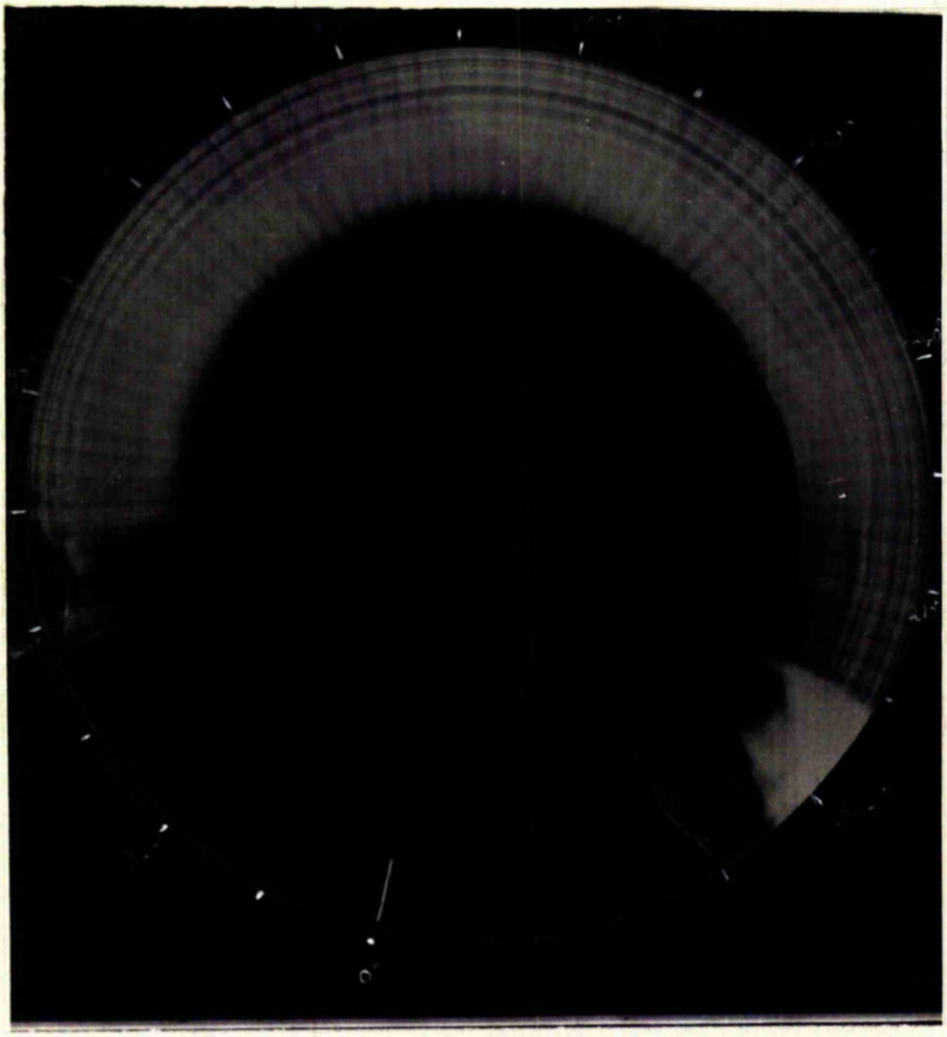
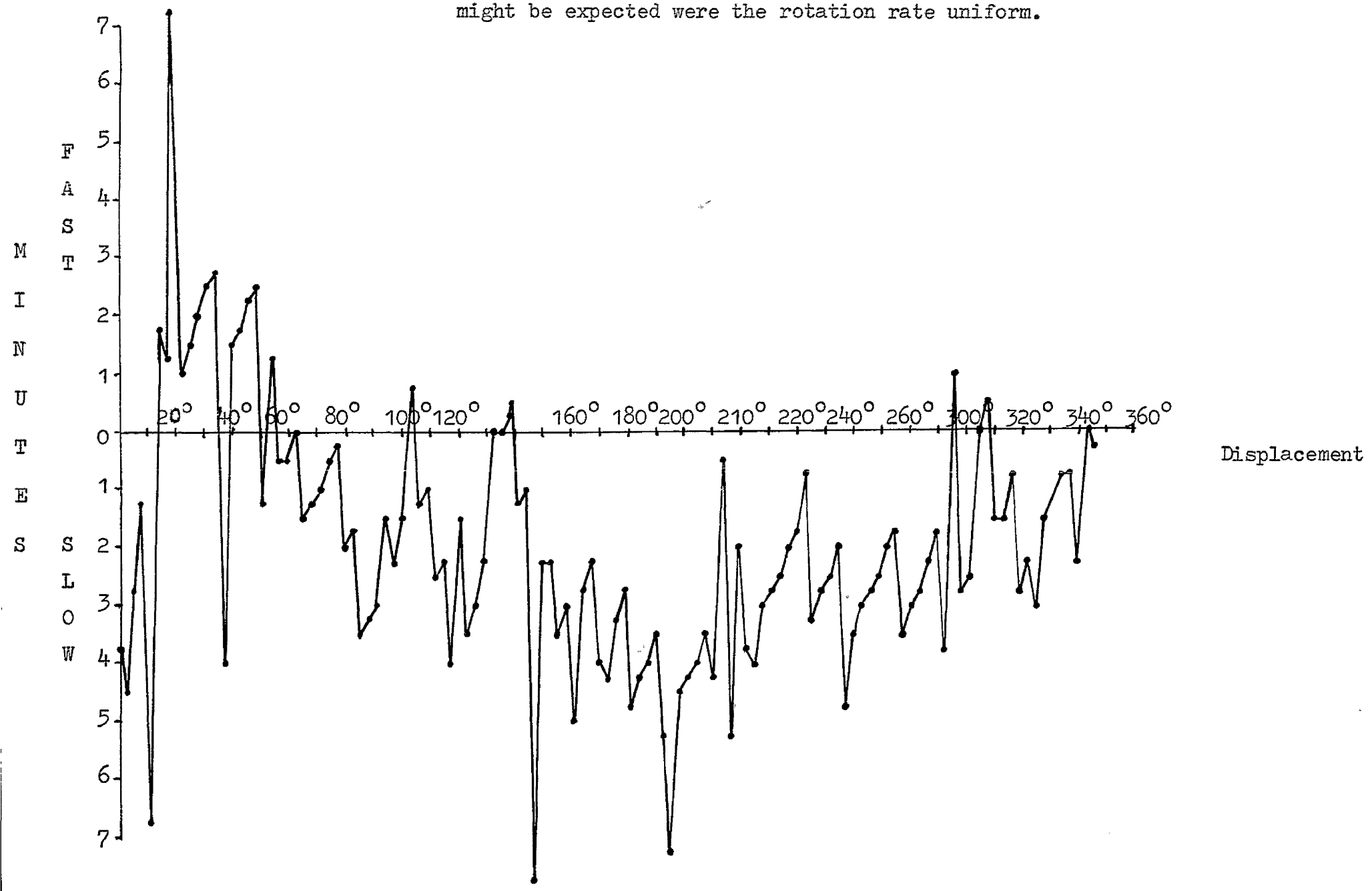


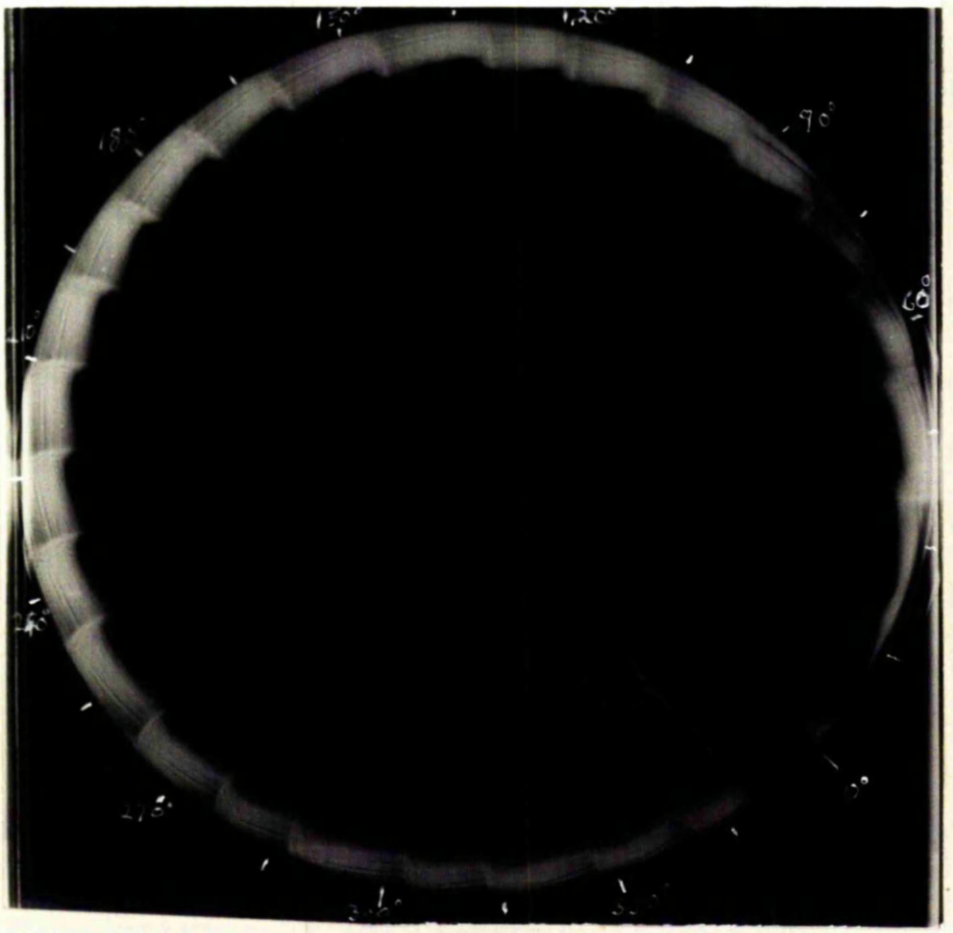
Figure 2. Clock (b).

Deviations from times at which particular displacements might be expected were the rotation rate uniform.



Photograph 3.

Spore pattern obtained with clock (b).

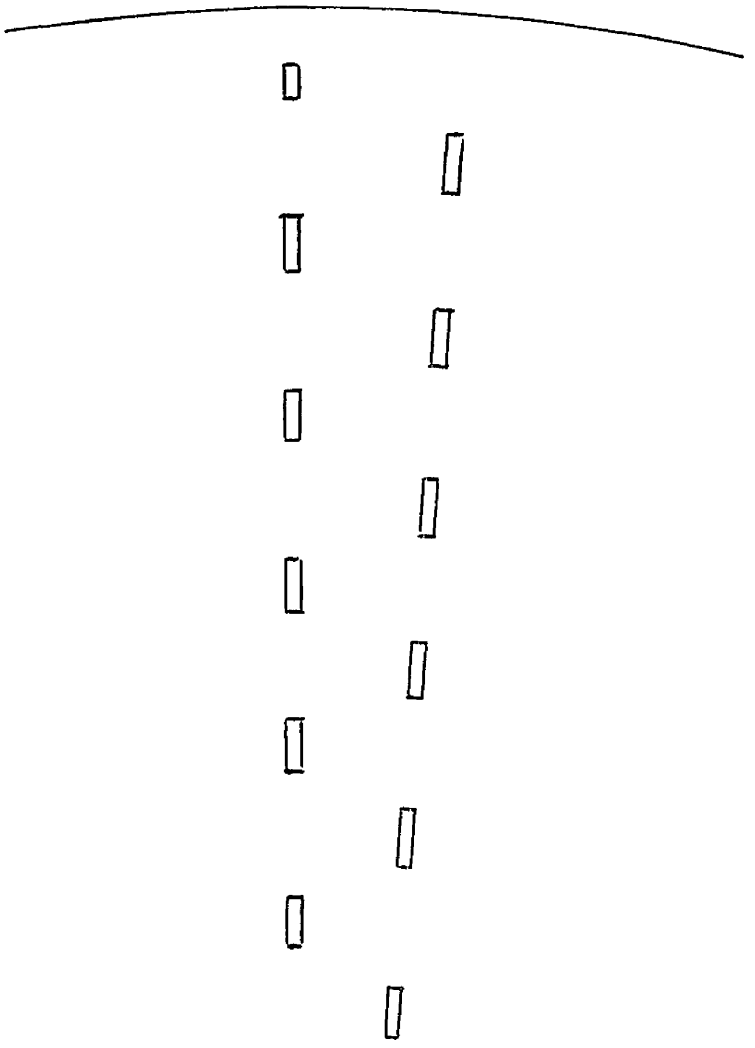


or behind time at particular displacements were the rotation rate uniform. Photographs 2 and 3 show the corresponding spore patterns on the glass discs. The irregularities in rotation rate in each case are similar and rarely exceed a gain or loss of more than 2 minutes (one half degree) in any hour (fifteen degrees). In neither case do the irregularities follow a regular sequence. The spore patterns are dissimilar, but each pattern is regular in itself. The irregularities in rotation rate of the order of ½ a degree or less per hour are insufficient to result in the considerably greater variation in spore pattern. Even in photograph 3, where the spore pattern shows a 15 minute period, no correlation is possible for there are frequent instances where the rotation rate has remained quite steady over periods of as much as one hour.

It is concluded from these results that the irregularities in spore pattern cannot be correlated with irregularity in the rotation of either clock mechanism.

Figure 1.

Arrangement of slits in mask.



x 2.

Appendix F

Uniformity of Spore Deposition Patterns from Different Parts of the Fruit Body of Trametes gibbosa

The obliqueness in the spore patterns obtained (Cay, Hutchinson and Taggart - appendix H) was interpreted as indicative of sporulation waves. If this interpretation were correct, it was to be expected that the patterns obtained from the hymenium above adjacent radial slit would be out of phase, unless the sporulation waves moved strictly radially to the collecting disc. This hypothesis was tested by experiments in which the single slit was replaced by a series of short slits on adjacent radii.

Material

Two fruit bodies were used, one from Rosneath and one from Kilcreggan (V.C. 99).

Apparatus

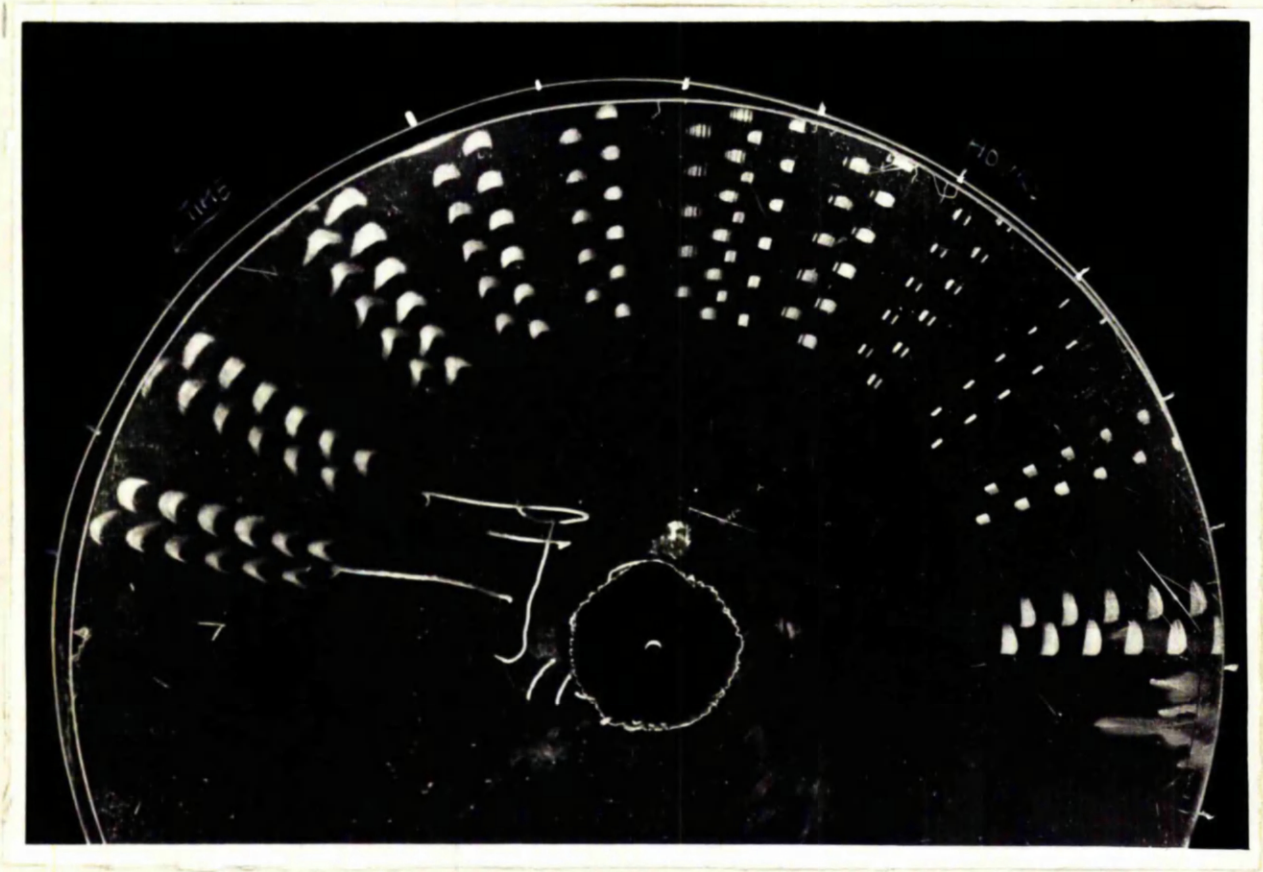
The spore collector previously described was used with modification of the slit. A sector was cut from the upper cover and this was replaced by heavy celluloid in which were cut two series of 6 small slits on separate radii (figure 1). The slits of the one series alternated with those of the other and measured 1 x 4 mm. Two of these masks were used, one with the slit series separated by 4° and one with them separated by 8°.

The disc was turned by clock (a) (see Part III) at one rev./day.

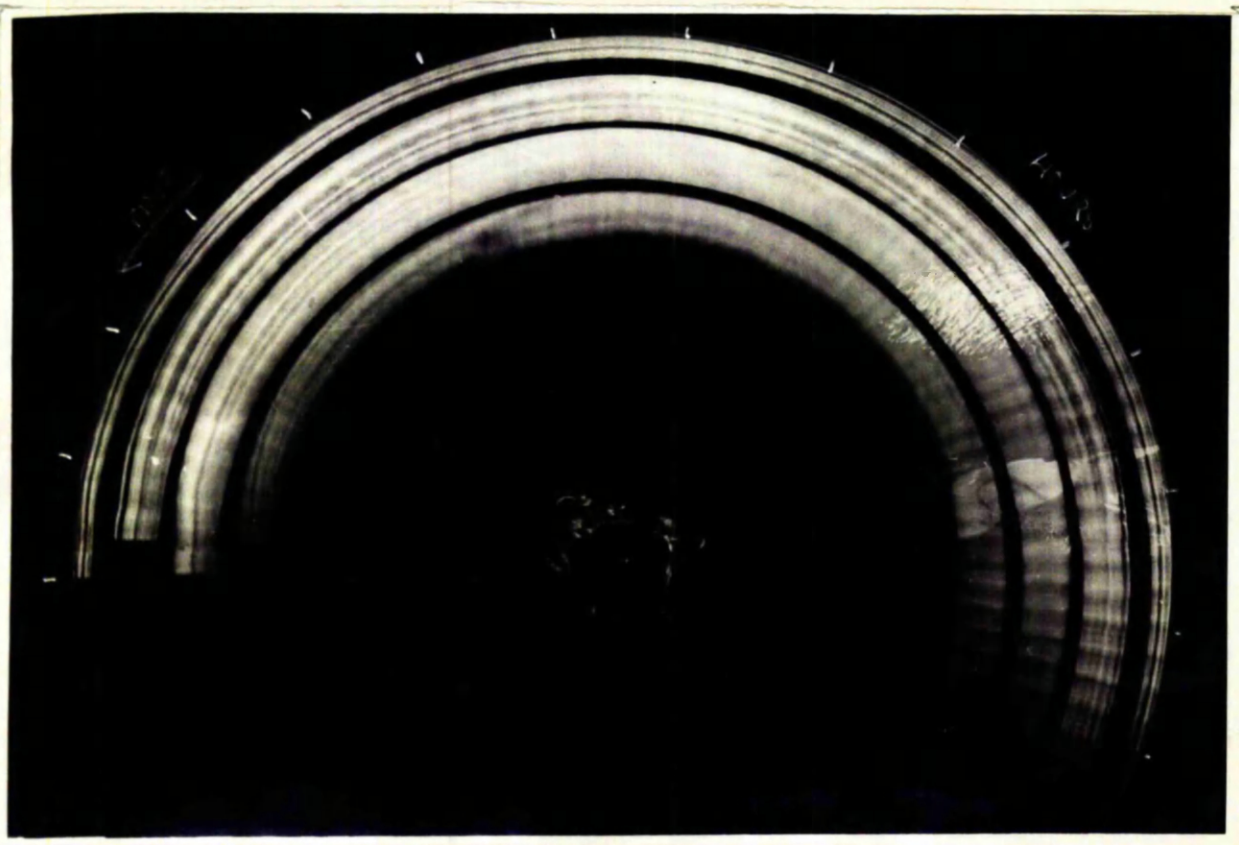
All eight experiments with the Rosneath fruit body were made in incubator A (see Part III) and all four with the Kilcreggan fruit body in

Spore patterns obtained with multiple slits.

Photograph 1. Incubator A 4° slit separation.



Photograph 2. Incubator B 4° slit separation.



incubator B. Both incubators were set at 20°C. Each mask was used with each fruit body.

Results

A typical result is shewn in photograph 1. The deposits in this spore pattern clearly fall into two groups corresponding to the two series of slits. They are separated by precisely 4°, that is by the separation of the two slit series in the mask. All the other experiments shewed a separation between the two series of deposits corresponding precisely to the separation of the two slit series in the mask used. The conclusion is that the spores in each series of deposits have been deposited at the same rate at the same time.

It is noted that the interval between successive dense zones in the deposits in photograph 1 is of the order of 60 minutes. Such an interval was found consistently in the experiments in incubator A. However, the spore patterns in incubator B (photograph 2) shewed intervals of the order of 15 minutes. Two simple slit experiments with the same fruit body in incubator B also gave 15 minute patterns. The possible significance of this different order of interval is discussed in Part III.

Conclusion

It is concluded that, if the spore patterns reflect sporulation rhythms, such rhythms must be synchronous over the whole fruit body. If this conclusion be applied to the previous work, the suggestion is that the interpretation of the obliqueness of patterns as explicable by sporulation waves must be wrong.

Appendix G

Spore Patterns produced by Species other than Trametes gibbosa

Trametes gibbosa was selected largely by chance for the work on sporulation rhythms. After completion of the work described in appendix H, it was thought desirable to see whether some more convenient species, producing similar spore patterns, might be found for further investigations. The principle disadvantages of Trametes gibbosa as an experimental tool had been found to be its complex distribution of hymenium and its local rarity. Its advantages included the retention of a very vigorous sporulation rate in the laboratory for a week or more.

The following common species were selected for examination:

Polyporus betulinus Fr.

Serpula lacrimans Pers. ex S.F. Gray.

Stereum hirsutum (Fr.) Fr.

Auricularia auricula-judae Schroet.

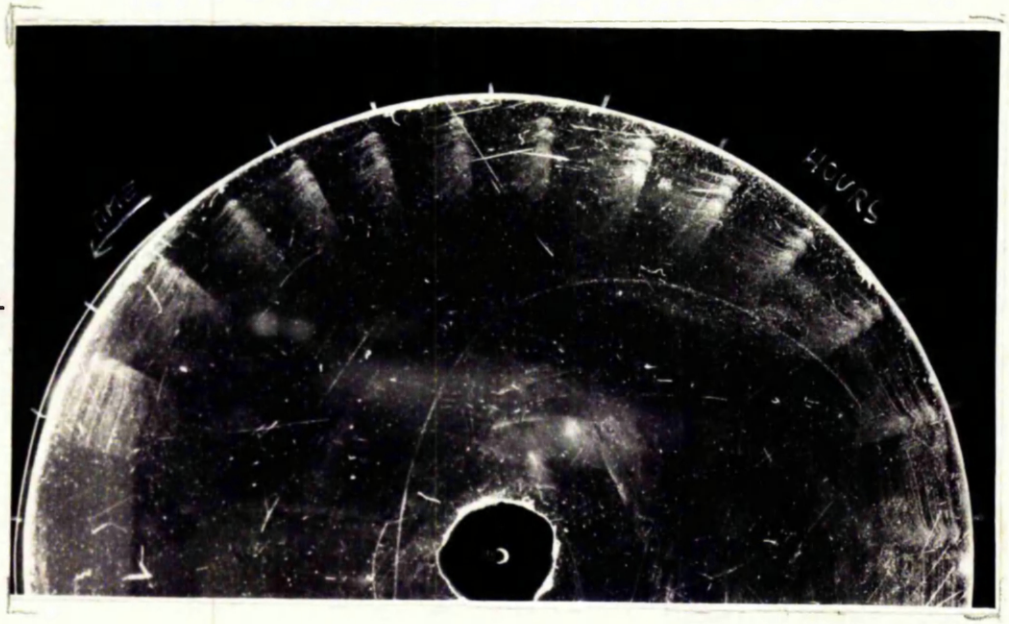
Of these species it was soon realised that the very precise orientation required for effective spore liberation from Polyporus betulinus (Part II) made the fruit bodies very awkward to handle. Work on this species was therefore discontinued after two preliminary experiments; the other species were examined by the following method.

Method

The apparatus used to record spore deposition from these species was that previously described (Gay, Hutchinson and Taggart - appendix H). The water cooled 20°C incubator (A) was that used in the previous work. The

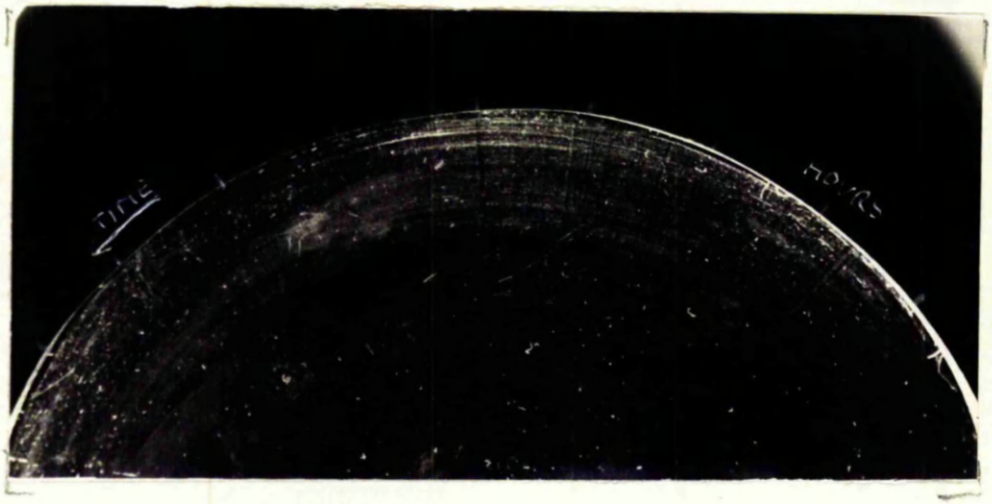
Photographs 1 - 3 .

Spore deposits from Serpula lacrimans and Auricularia auricula-judae.



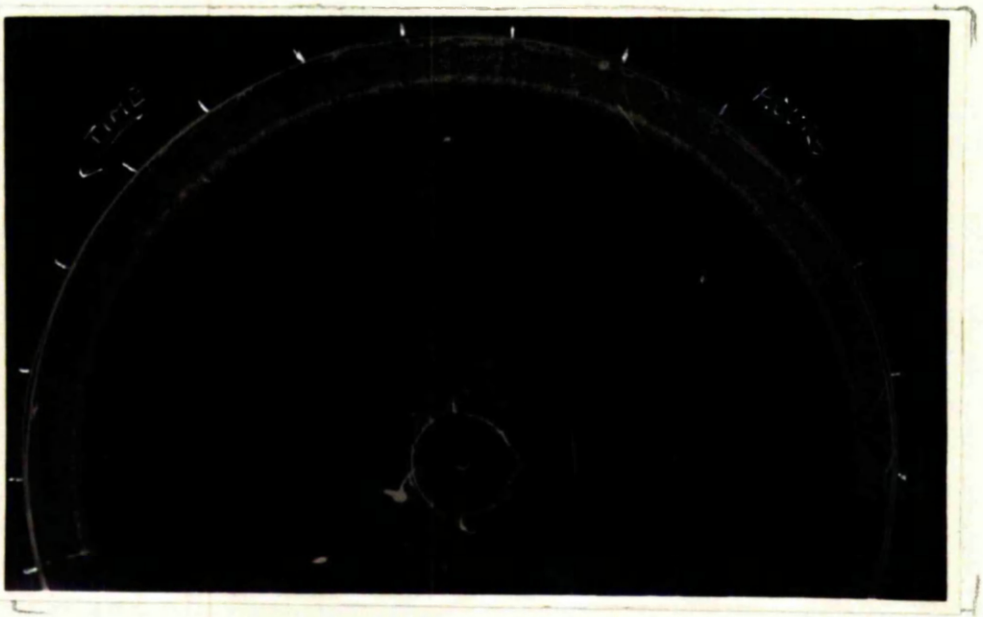
Photograph 1.

Serpula lacrimans.



Photograph 2.

Serpula lacrimans.
A uniform deposit.

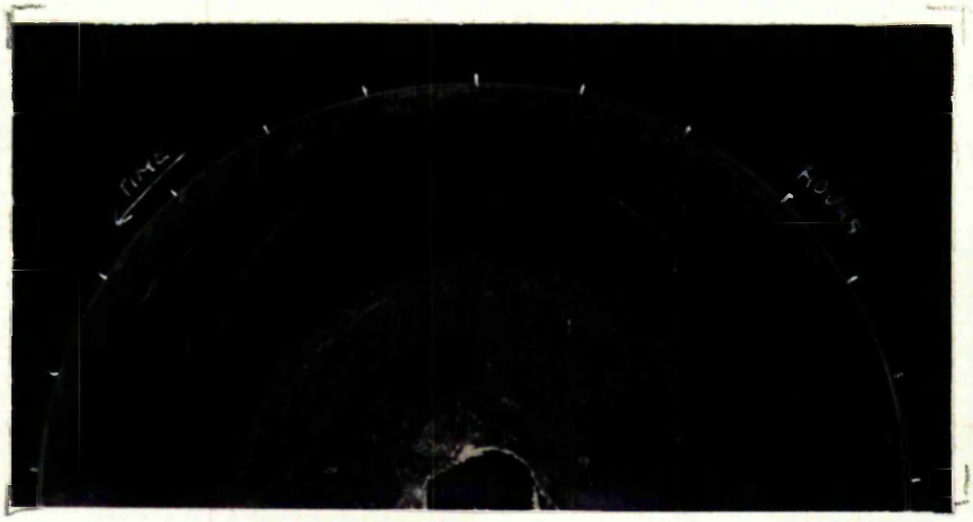


Photograph 3.

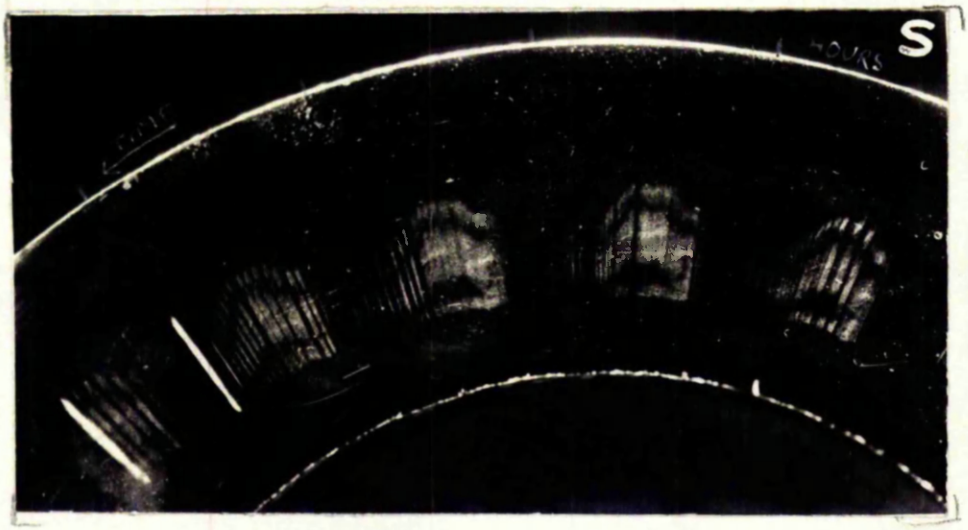
Auricularia auricula- judae.

Photographs 4 - 6.

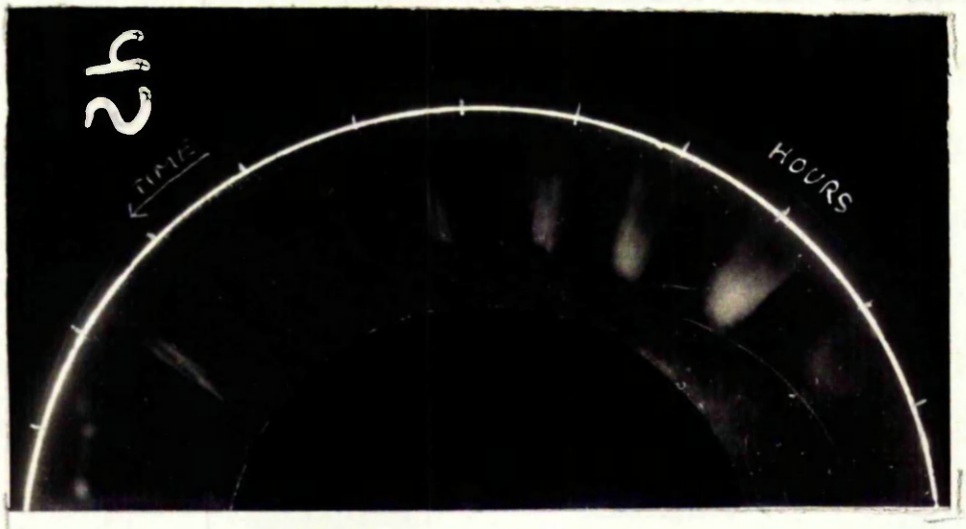
Spore deposits from Auricularia auricula-judae and Stereum hirsutum.



Photograph 4.
Auricularia auricula-judae.



Photograph 5.
Stereum hirsutum.



Photograph 6.
Stereum hirsutum.

glass disc was turned at one revolution per day by the spring driven clock (a) previously used. In all experiments as much of the fruit bodies substrate was retained as possible. Fruit bodies used in successive experiments were left for the few intervening hours in the incubator with the door open. For longer periods they were kept in a damp tank in the laboratory. A thermograph record of incubator temperature beside the fruit body was obtained during most experiments.

Results

Examples of the spore patterns obtained are given in photographs 1 - 6. These, together with the features making particular species suitable for further work, are summarised in Table I.

Discussion

(I) Selection of Material for Further Work

The object of these investigations was to find a species more convenient for further work. However on consideration of the points given in Table I *Trametes gibbosa* itself was thought to be the most useful species for this purpose. It was hoped to overcome the local rarity of the species by the artificial culture of fruit bodies (appendix D). The disadvantages of its complex hymenium were considered to be outweighed by its advantages. In the choice of *Trametes*, it was also borne in mind that the further investigations were intended to clarify the interpretation of the results already obtained. Thus it was thought undesirable to use a species with spores of a different order of mass or volume in investigating the explanation of a pattern depending on gravitational settling.

Table I. Suitability of *Trametes gibbosa*, *Serpula lacrimans*, *Stereum hirsutum* and *Auricularia auricula judae* for further experimental work.

Species	*Trametes gibbosa*	*Serpula lacrimans*	*Stereum hirsutum*	*Auricularia auricula-judae*
No. Experiments	15	4	6	7
Period in Spore Pattern	1-1½ hrs.	¼-1½ hrs. (once zero)	c. 1 hr.*	¼ - 1 hr. (once zero)
Availability	Scarce	Moderate	Good	Very Good but seasonal
Longevity in Lab. conditions	Very Good	Poor	Reasonable	Poor
Distribution of hymenium	Complex	More or less simple	Simple	Simple
Convenience for Handling	Convenient	Awkward (Separates from substrate)	Convenient	Convenient
Suitability of spores for photography	Very suitable	Poor	Suitable	Very suitable
Vigour of sporulation	Very Vigorous	Good	Poor	Reasonable
Order of spore ** volumes taking *Trametes* spores as unity	1	13	3½	35

* A series of radial deposits within the major area with periods of about 3 minutes were twice present (e.g. photograph 5).
** Based on spore sizes given by Wakefield and Dennis 1948.

(II) Interpretation of the Spore Patterns

On the whole the spore patterns were similar to those which had been found for Trametes gibbosa, but subsequent investigation (Part III) has shewn the latter to be primarily controlled by the operation of the incubator heater. Thus nothing can be concluded from these patterns. It is, however, of interest that patterns with a much shorter period (c. 15 minutes) were obtained on several occasions with Serpula lacrimans and Auricularia auricula-judae. On two occasions Stereum hirsutum gave peculiar 'organ pipe' patterns within the major deposit (photograph 5). We can draw no definite conclusion as to the significance of these short period rhythmical patterns, but it is possible that they may be of similar origin to the 15 minute rhythms found in the subsequent work with Trametes gibbosa. (Part III).