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The Action of Light on Bacteria. III

H. Marshall Ward

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XX. *The Action of Light on Bacteria.*—III.

By H. Marshall Ward, *D.Sc., F.R.S., Professor of Botany, Cooper's Hill.*

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[Plate 87.]

Downes and Blunt,* the first who observed the destructive action of light on bacteria, had already raised the question as to which of the various rays of the spectrum are active, and they made some experiments with coloured screens. The great objection to all their results is that they did not use pure cultures. Arloing,† Janowsky,‡ Geisler,§ and Kotljar‖ employed pure tube cultures—broth, gelatine, or potato—and exposed them behind various screens, endeavouring to judge of the action of the light by the relative rapidity with which the broth becomes turbid, or at which the masses of colonies spread over the surface of the solid media, &c.

The chief objection to these methods is that it is very difficult to compare and contrast two separate culture-tubes of this kind, though it should be noticed that Janowsky employed a very ingenious expedient for ensuring at least that his broth-cultures should be exactly similar to start with.

That the light of the electric arc also acts bactericidally was first shown, I believe, by Arloing¶ in 1887, and has been more closely studied by Geisler** and Chmelewsky,†† but in addition to the criticism already made on their methods, they failed to obtain the most satisfactory results, owing to their use of glass instead of quartz.

The same criticisms apply to various attempts made by these observers to estimate the effects of different regions of the spectrum by putting their separate tubes into

* 'Roy. Soc. Proc.,' 1877 and 1878.
† 'Comptes Rendus,' 1885.
‡ 'Centr. f. Bakt.,' 1890.
§ 'Centr. f. Bakt.,' 1892.
‖ Wratsch, 1892 (abstract in 'Centr. f. Bakt.,' 1892).
¶ 'Comptes Rendus,' 1887.
** 'Op. cit.,' 1892.
†† 'Centr. f. Bakt.,' 1892.

the various regions. Moreover, the conclusion reached by these different observers as to which rays are efficacious and which not differ. ARLOING failed to distinguish which rays are the more effective; and JANOWSKY concluded that the red rays, as well as the blue-violet, are more effective than the yellow ones. SANTORI* concluded that neither the red nor the violet rays are the active ones. GAILLARD† concluded that all the rays of the spectrum are active.

During the past year I have made a large series of screen experiments by allowing the light of the sun to fall on circumscribed areas‡ on Agar-plates arranged in various ways and covered by the screens of which the action is to be compared. The following are only a few of those I propose to publish in a more detailed form subsequently.

In order to render more intelligible the explanations I have to offer for some of the phenomena to be described, it may be well to examine somewhat closely the behaviour of one of these plates during the growth of the colonies which result from the unexposed, or only partly exposed, germs, while incubation is going on.

The following series of photographs were taken from a plate exposed to direct bright sunlight for 3 hours, on March 27—*i.e.*, from 12.15 to 3.15 P.M., and covered with a stencil letter Y.

After about 18 hours' incubation at 20–22° C. it is generally possible to make out the dim but blurred outlines of the letter, but the contrast between the clearer area corresponding to the letter and the as yet only slightly more opaque parts of the Agar protected from the sun—slight, because the colonies have not yet had time to develop far enough to render these parts opaque—is, as yet, too slight to enable one to photograph the letter.

After 24 to 30 hours, however, a photograph can usually be taken. Fig. 1 was taken after 24 hours, and we see that the letter is not yet quite sharp at the edges and appears too large. This phenomenon is apparently due to the rays reflected inside the plate being still powerful enough to slow, but not to totally prevent the germination and development of the spores at the edges.

The next figure (fig. 2) shows the letter sharp and clear after 24 hours' longer incubation, time having now been given for these marginal colonies to develop out.

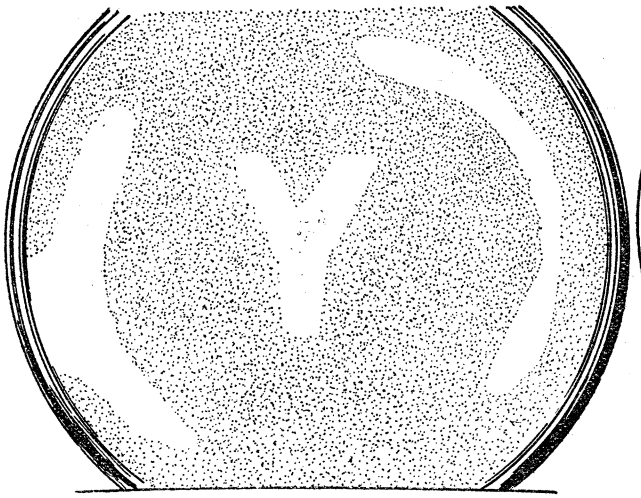
Now comes in the second phenomenon I wish to call attention to. After a further 24 hours' (fig. 3) incubation we notice small colonies on the otherwise cleared area of the letter becoming more and more conspicuous. These colonies were already present in the earlier figure, and I explain them as developed from germs which have

* Quoted by GEISLER, *loc. cit.*

† 'Thèse de Lyon,' No. 396.

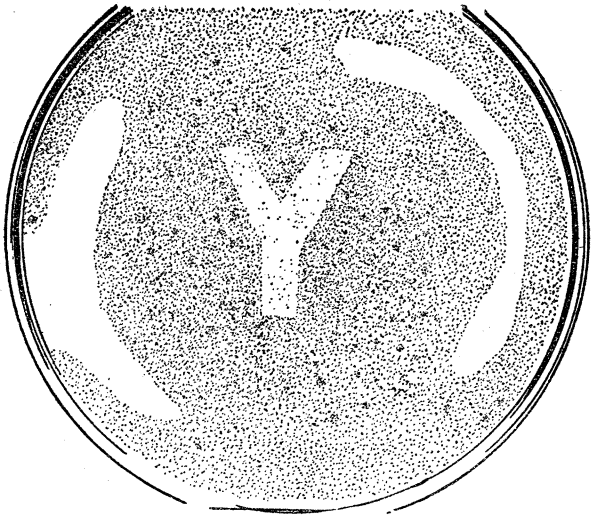
‡ The credit of first publishing the application of the principle of determining the light-action by incubating an agar-plate partly exposed to the sun and partly sheltered from it is due to BUCHNER ('Centr. f. Bakt.,' 1892), but I had not only long employed this method, but had also thus applied it before seeing BUCHNER'S paper. So far as I know no one but myself has used coloured screens in combination with this method.

Fig. 1.



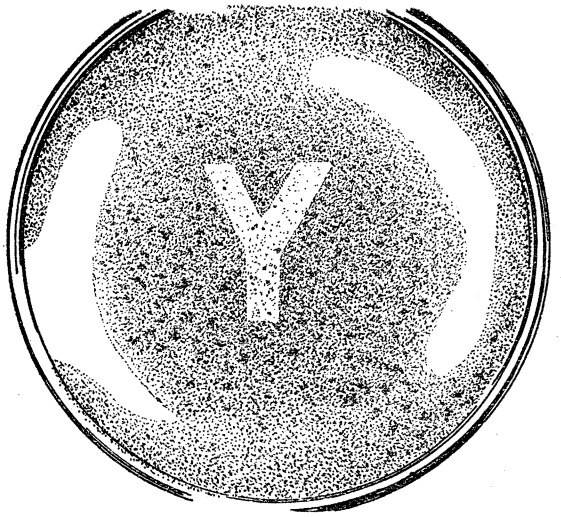
24 hours' incubation.

Fig. 2.



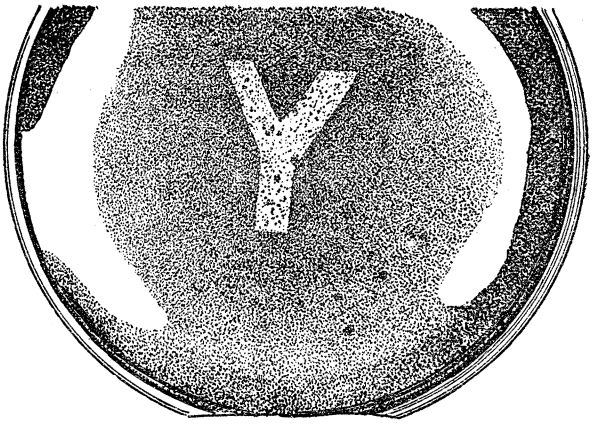
48 hours' incubation.

Fig. 3.



72 hours' incubation.

Fig. 4.



96 hours' incubation.

Figs. 1–4. An agar-plate of anthrax spores exposed behind a stencil-plate Y from 12.15 to 3.15 p.m. on March 27, and then incubated at 20–22° C. and photographed at intervals. The photographs were taken as transparencies, against a N. window. The two crescentic clear areas are due to the agar-film not completely covering the plate in this case.

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escaped the action of the light. How? There are several possible explanations—(1) The light was not sufficiently intense, or did not act long enough, to kill certain spores more resistant than the majority; (2) The spores from which these colonies originate were so deeply submerged that the oxidising action set up by the light could not take place freely; (3) These submerged spores lay so exactly behind a number of other spores that the latter partially screen them from the light.

If it were not for the fact that these spores *do* eventually germinate out freely, and thus show that the agar is still a fit medium for their growth, it might also be suggested (4) that these spores have escaped suffusion by some poisonous substance developed in the medium; but I have given such excellent evidence of other kinds against this position that I cannot accept it as probable.

On the whole, the most rational explanation seems to be the third—these later developing colonies are from spores partly screened by others, living or dead, which intervened between them and the incident rays.

The following series of photographs illustrates at the same time the comparative action of certain glass screens, and some phenomena already met with when incubating the plates.

The plate, a large glass one, was covered by a square cardboard screen, in which five circular holes were pierced. The corresponding five circular areas are visible in fig. 5.

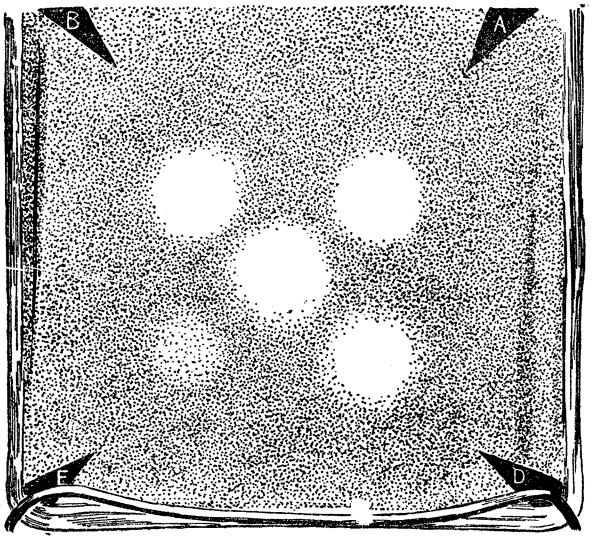
The central hole was covered with a piece of ordinary window glass, that to the right above was left uncovered; consequently the light through the latter traversed only the glass of the plate, that through the former passed through two plates of glass. The hole to the left above was covered with a piece of deep blue glass; that to the right below with a piece of pale blue glass, and that to the left below with a piece of a peculiar brownish-purple glass which cut off much of the blue and violet rays—all the others let most of the blue-violet pass readily.

The plate thus screened was exposed to direct bright sunshine on April 5th, from 11 A.M. to 2.30 P.M.—*i.e.*, $3\frac{1}{2}$ hours, and was then put into the incubator at 20° C.

After $18\frac{1}{2}$ hours' incubation the photograph fig. 5 was taken, showing that some light action was evident on all the circular areas. As incubation proceeded it became evident that (1) the action was unequal on the various areas, (2) that it was least intense on the area covered by the brown-purple glass, and most intense on that left uncovered, (3) that a peculiar halation effect is visible at first, rendering the outlines blurred, but disappears later, and the circles become sharp and clear, and (4) that not all the germs on the areas are killed, for a few colonies appear later (after three or four days) even on the clearest area. (*Cf.* the sequence of the photographs.)

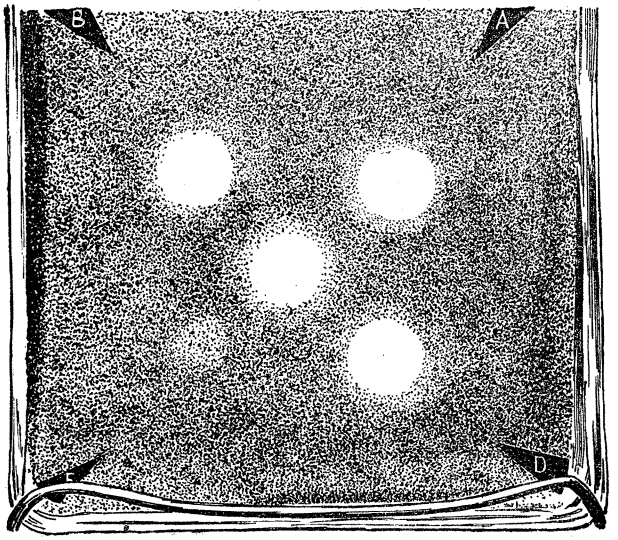
The peculiar halation effect I explain as follows. The light which enters the circular areas is partially reflected from the surfaces inside the plate, and even these reflected rays are powerful enough to inhibit the growth of the colonies at the

Fig. 5.



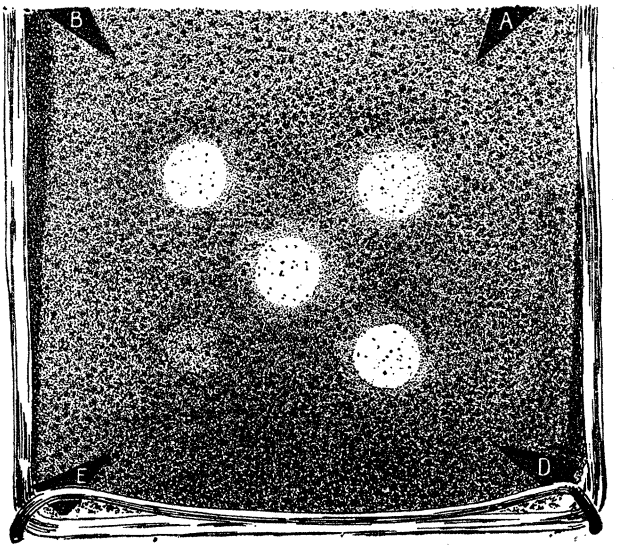
$18\frac{1}{2}$ hours' incubation.

Fig. 6.



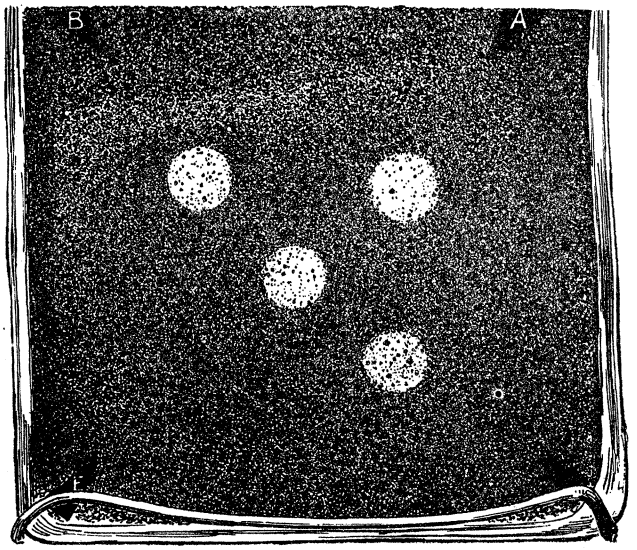
25 hours' incubation.

Fig. 7.



42 hours' incubation.

Fig. 8.



4 days' incubation.

Figs. 5–8, Plate of agar, with anthrax spores, covered by a square screen with five circular windows with coloured glasses (see p. 964), and exposed for $3\frac{1}{2}$ hours on April 5th, and photographed as transparencies at various stages of incubation as indicated.

margins for a short time; later on, however, these colonies also develop out normally and so sharpen up the edges.

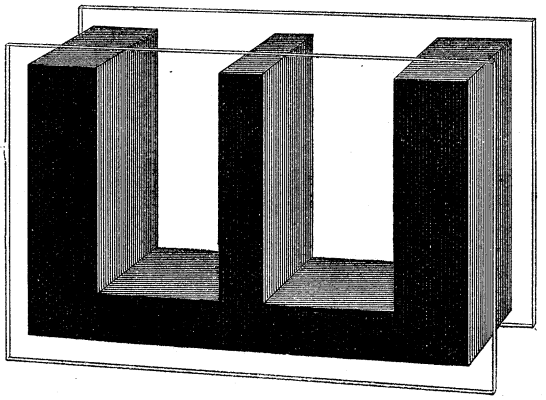
The appearance at a later date of colonies on the apparently cleared areas, I take to be due to the partial protection of some of the spores by others lying serially over them in the line of the incident rays of light, and so screening them more or less from these rays. Even this protection does not prevent the light from slowing the development of these germs, however, though they are able to develop later on.

These phenomena afford additional proof that the action of the light is direct, and that the agar on the illuminated areas is not rendered unfit for the development of the colonies.

What precedes, will have made intelligible some of the difficulties to be encountered in attempting to discover what may be called the quantitative results of the exposures to sunshine. It occurred to me, however, that more might be done if different parts of *the same plate* were exposed simultaneously behind different screens.

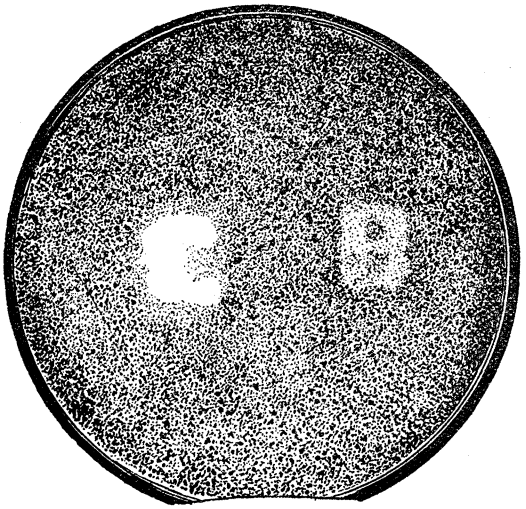
To carry out this idea still further, I made a number of double ebonite cells after the following pattern. A solid block of ebonite, about $\frac{3}{4}$ inch thick, 3 inches long, and 2 inches broad, is cut into the shape of a Roman capital E, and a plate of good, thin, flat, clear glass cemented to each side as in fig. 9. I obtained an excellent glass

Fig. 9.



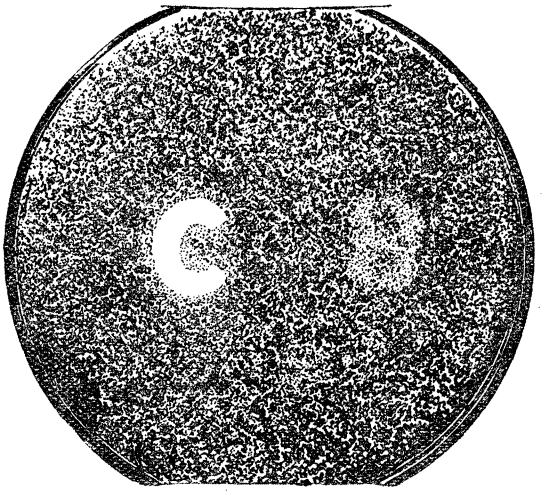
from Messrs. Chance, of Birmingham, for this purpose, and after trying many kinds of cement, found that, on the whole, gold-size held the best, if the cemented cell is placed in a hot oven for a short time, and then allowed to cool under a weight; whenever one of these cells took to leaking, it was easy to repeat this heating and pressing and make it water-tight again. Of the two compartments into which these cells are thus divided, I filled one with filtered distilled water, and used it as a standard; the other was filled with some other solution, and by not allowing the level to be the same in the two cells, it was easy to ensure that no leakage occurred from one compartment into the other during the experiment.

Fig. 10.



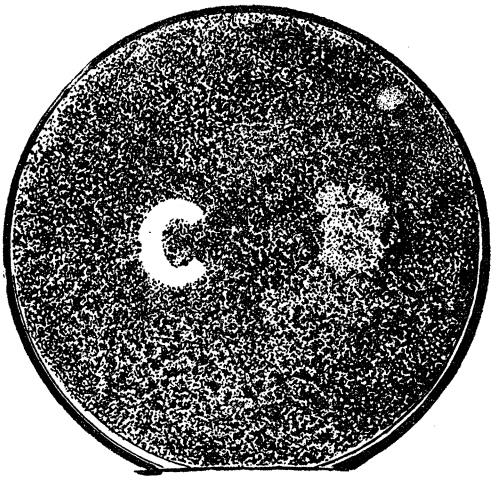
19 hours' incubation.

Fig. 11.



43 hours' incubation.

Fig. 12.



111 hours' incubation.

Figs. 10–12. Exposed 12–4, March 30. *B* = behind *æsculin*; C = behind *water*, screens equal thickness.

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It should be borne in mind that the depth of water, or watery solution to be traversed, was here considerably more than in previous experiments, a fact of significance where such a solution as ammoniacal $CuSO_4$ was employed; the image of the bright sun was scarcely visible through such a screen, whereas the eye could not bear the light traversing the corresponding screen of K. bichromate.

The following series, one of a large number of such comparative experiments, will serve very well to illustrate the value of this method of examination. (Figs. 10–12.)

The photographs are those of a plate exposed for 4 hours—from 12 noon to 4 P.M.—on March 30, to the direct rays of a bright sun. The plate was covered with two stencil letters, B and C; the B behind a strong solution of æsculin, in water, the C behind pure water only.

After 19 hours' incubation the difference in the light-action is already evident. The rays passing through the water were so strong, as not only to clear the area of the C, but to produce the marked blurring effects already explained, whereas those passing through the æsculin, being poor in violet and blue-violet rays, have only partially cleared the B-shaped area, and the reflected ones had not enough power to produce any conspicuous blurring. After 5 hours' further incubation the blurring at the edges of the C is already diminishing, but the interval is too short for more marked developments. After 43 hours (fig. 11), however, we notice very great progress. Not only is the contrast much more pronounced, but the C is evidently very effectually cleared; whereas there are so many spores on the B which have escaped the light action, that their developing colonies are rapidly obscuring the letter. This is still more evident after 111 hours' incubation. Later on the B was obliterated altogether, and only two small colonies had escaped on the C.

The marked effect through the water is unquestionably due to the difference in the quality of the blue-violet light which has traversed the screen, and not to any difference in the heating effects, for in this respect the screens are equal, as each offers the same thickness of water and glass to the light.

I have evidence, deduced from experiments with alum and with rock-salt crystals used as screens, however, which goes to show that other things being equal, the access of more heat rays enhances the light effect—indeed, we should expect that, on almost any hypothesis as to the *modus operandi* of the rays on the cells.

The following Table summarises at a glance the data and results of a number of these comparative screen experiments:—

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Experiments with Double-celled Thick Screens.

Number, &c., denoting plate.	Letter or figure used.	Date made.	Nature of screen.	Date exposed.	Time of exposure.	When put to incubate.	Period of incubation at 25° C.	Time letter, &c., visible.	Results.	Remarks.
II.*a*.	B	Mar. 10	Alum	Mar. 10	1.30 to 3.30 P.M.	4 P.M., Mar. 10	7 d.	20 h.	The C was sharper and cleaner than the B	Good hot sun and blue sky, therefore alum may be taken to stop a *little* of the action?
II.*b*.	C	,,	Water	,,	,,	,,	,,	20 h.		
8.*a*.	B	Mar. 13	Water	Mar. 13	1.45 to 4.15 P.M.	4.30 P.M., Mar. 13	4 d.	40 h.	The B just visible, but C never appeared	Hazy and cloudy, especially after first hour, hence little blue; therefore quinine inactive, because too feeble to get through
8.*b*.	C	,,	Quinine	,,	,,	,,	,,	..		
13 (1).	O	Mar. 17	* Glass and glass	Mar. 17	1.45 to 4 P.M.	4.30 P.M., Mar. 17	8 d.	40 h.	Least clear of all at first, then clearest	* The screen here was the two glass faces of the cell, with $\frac{3}{4}$ in. air-space between
13 (2).	O	,,	* ,,	,,	,,	,,	,,	,,	As 13 (5) at first, then = second clearest	
13 (3).	O	,,	†	,,	,,	,,	,,	,,	A mere blurred area	† This window was partly covered by liquids and partly open
13 (4).	O	,,	Quinine	,,	,,	,,	,,	,,	Clearest and sharpest at first, finally least so	Bright blue sky alternating with large silvery clouds: high wind, cool. The areas exposed were 5 circular holes $\frac{1}{4}$ in. diameter
13 (5).	O	,,	Water	,,	,,	,,	,,	,,	Less cleared at first than 13 (4). Finally = third clearest	
14.*a*.	H	Mar. 18	$CuSO_4Am$	Mar. 18	1 to P.M.	4.30 P.M., Mar. 18	,,	$40\frac{1}{2}$ h.	N more cleared than H, but both have some colonies at last	Very bright clear sun, but air cool. H sharper than N at first, but on 21st reversed
14.*b*.	N	,,	Water	,,	,,	,,	,,	,,		
A.1.	K	Mar. 21	Water	Mar. 21	11.30 to 3 P.M.	3 P.M., Mar. 21	6 d.	..	No distinct letters, only clear areas, roughly in outline of letters	Very hot bright sun and blue sky. Over exposed, and internally reflected rays cleared beyond edges of letters
A.2.	Z	,,	Æsculin (strong)	,,	,,	,,	,,	..		
I.*a*.	H	Mar. 24	Quinine	Mar. 25	10.30 to 3.30 P.M.	4 P.M., Mar. 25	..	..	Negative	The inhibition and destruction may have been caused by heating too much when pouring the plates?
I.*b*.	N	,,	Water	,,	,,	,,				
II.*a*.	C	,,	Æsculin (strong)	,,	,,	,,				
II.*b*.	B	,,	Water	,,	,,	,,				

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EXPERIMENTS with Double-celled Thick Screens (continued).

Number, &c. denoting plate.	Letter or figure used.	Date made.	Nature of screen.	Date exposed.	Time of exposure.	When put to incubate.	Period of incubation at 25° C.	Time letter, &c., visible.	Results.	Remarks.
X.*a*.	H	Mar. 27	Quinine	Mar. 27	12–3	3 P.M., Mar. 27	4 d.	18 h.	The N far sharper and clearer than H, and former very nearly cleared on 29th	Very hot sun, hazy first hour, then brilliant. The H gradually obliterated
X.*b*.	N	,,	Water	,,	,,	,,	,,	18 h.		
XI.1.	B	,,	Æsculin (strong)	,,	,,	,,	,,	26 h.	The C was seen as a clear square patch at 18th h., but not out clear till 26th, when B was obliterating	
XI.2.	C	,,	Water	,,	,,	,,	,,	18 h.		
a.1.	T	,,	K. bichromate	,,	,,	,,	,,	..	No trace of T. The X came out normally and clear	
a.2.	X	,,	Water	,,	,,	,,	,,	18 h.		
β.1.	E	,,	K.chromate (dilute)	,,	,,	,,	,,	18 h.	A very faint ghost of E appeared, and slowly obliterated after 26 h. W sharp and clear	The chromate was in dilute solution, and allowed a considerable amount of blue to pass
β.2.	W	,,	Water	,,	,,	,,	,,	18 h.		
γ.1.	B	,,	Dilute fuchsin	,,	,,	,,	,,	18 h.	The C far sharper than the B after 24 hours	The dilute fuchsin allowed a large amount of violet to pass
γ.2.	C	,,	Water	,,	,,	,,	,,	18 h.		
δ.1.	M	,,	Strong $AmCuSO_4$	,,	,,	,,	,,	18 h.	The M came out sharper than the Z at first: then both clear	
δ.2.	Z	,,	Water	,,	,,	,,	,,	18 h.		
III.*a*.	P	,,	Quinine	,,	,,	,,	,,	26 h.	Both letters come out, but the P decidedly the sharper	
III.*b*.	N	,,	Æsculin	,,	,,	,,	,,	26 h.		

It is evident, from the foregoing experiments that, for *Bacillus anthracis* in agar, at any rate, the bactericidal action of the various rays of the spectrum is *nil* in the red, orange, yellow, and true green. It begins somewhere near the line F, and extends right on into the violet; whether it goes further—into the ultra-violet—could not be determined by these experiments, since the glass and water-screens are impassible barriers to what little ultra-violet there is in the light employed.

The question next arises: where is the maximum effect? I made a special series of comparative exposures with the following solutions to try and determine this point, and have here to record my sincere thanks to Sir Gabriel Stokes for the trouble he took in giving me valuable information, and suggestions on the matter of choice of chemicals.

The solutions employed were æsculin, sulphate of quinine, K. chromate, picric acid, methylene blue in picric acid, and Prussian blue in oxalic acid, and a solution of K.K.S.—this latter kindly prepared for me by Professor McLeod, to whom I also owe thanks for several suggestions and much information.

The general results were that (1) in layers of the same thickness, both water and ammoniacal cupric sulphate allow more effective rays to pass, with equal exposures, than any of the other solutions. (2.) All the blue-violet rays are effective to some extent, for even methylene blue in picric acid and picric acid alone allow some active rays to pass, and these can only be the blue-green, and there is considerable action behind æsculin, which cuts off all the violet and some blue (Table, p. 969). (3.) The action is stronger through quinine sulphate and æsculin, especially the former, which cut off the violet end, than through K. chromate or any of the solutions which cut off the intense blue near the line G.

Consequently the bactericidal maximum appears to be somewhere in the neighbourhood of the line G, and, as will be shown later, this is fully confirmed by experiments with the spectrum.

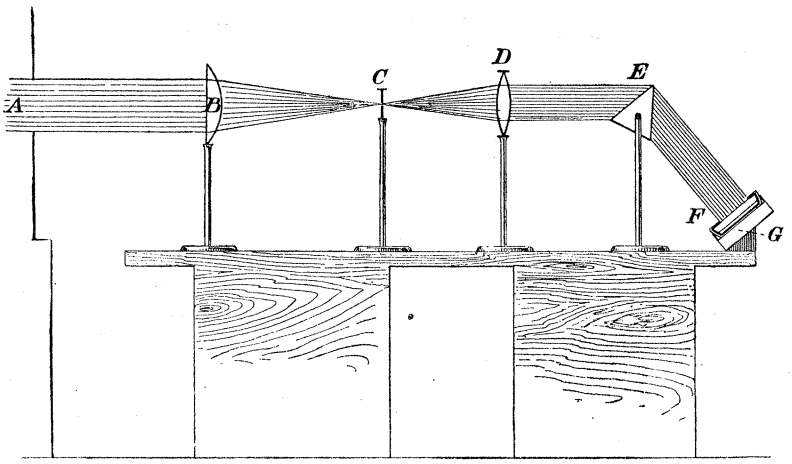
I have already stated that some preliminary attempts to obtain a record of the action of the various parts of the spectrum direct on the plates, were made during the winter of 1892. The spectra tried were those of the sun and of the electric arc, but the results were not decisive, beyond convincing me that the solar spectrum does act on the plates, and that the blue end shows more effect than the red end. Beyond this the matter seemed discouraging, and at one time I thought I should have to abandon the attempt to obtain records sharp enough to photograph.

I have since, however, obtained much better results, and now proceed to describe these experiments. I do not propose to catalogue all the difficulties and the numerous abortive and time-consuming failures which intervened before I obtained the satisfactory records to be referred to, and shall merely remark on such as bear on the practical solution of the problems as I proceed. Everyone who has worked with sunshine in our climate knows how often a promising morning ends with an overcast sky, and all bacteriologists will understand how a plate is spoiled under such circum-

stances. Enough, that failures may arise from the slipping of the agar films, over- or under-dosing them with spores, and a number of other points which experience only enables one to guard against. The time of exposure had also to be settled by trial and error, of course.

Taking the solar spectrum first, the method was as follows, 13 : A parallel beam (A) was obtained by means of a heliostat and plane silvered mirror, and thrown, through a window into a dark room, on to a large plano-convex lens (B); the slit, of metal, was

Fig. 13.



placed horizontally so that the focus of the condensed rays fell just beyond it (C), so that as much light as possible was made to traverse the slit. These rays were then gathered up and again rendered convergent by a double convex lens (D), and the beam thrown on to a prism (E) placed horizontally.

By these means I was able, during several days this summer, to obtain a fairly pure spectrum, which was very brilliant, although the slit was so narrow. This last is a factor of great importance, because the exposures necessary are considerably longer than with undecomposed light.

The prepared film was covered with a glass plate, in which was a slot, about three inches long and half an inch wide, instead of the ordinary glass lid. Over this slot was a plate of thin quartz. The whole plate, except the exposed slot, was protected with sterilised black paper, followed by tin-foil, and finally wrapped in stiff cartridge paper. (F, G: compare fig. 17, p. 977.)

The spectrum, after traversing the thin quartz-plate, fell direct on the surface of the film of agar with its embedded spores (F, G).

It is obvious that, provided I could keep all the various rays acting with sufficient intensity, for the necessarily longer period of exposure, on the same part of the prepared film, those which possess no bactericidal effect should leave the spores on which they fall unaltered, whereas those rays which kill the spores should register their effect, and similarly for those which only inhibit or weaken the spores.

This turned out to be the case, and scrutiny of the plate after being incubated for 24 to 48 hours shows an image (fig. 14), in the form of graduated clearer areas corresponding with the parts on which the active rays fell.

In fig. 15 the spectral photograph is marked with vertical lines showing the limits of the chief colours,* these lines rising from a horizontal one showing the length of the visible spectrum, and of the exposed slot.

Beginning at the left-hand, it is clear that the infra-red, the red, orange, and yellow are absolutely without effect, for the spores which were exposed to these rays have germinated out as well as anywhere on the non-exposed parts of the film. It is also seen that at least the less refrangible half of the green is without effect, and the action commences somewhere towards the limit between the green and the blue,—that is between the Fraunhofer's lines E and F—and increases in intensity as we pass up the blue towards the violet, as shown by the relative clearness of the area on which the spores have been killed.

Passing into the violet itself, we find the action soon becomes feebler, as shown by more and more spores having germinated out, and ceases about halfway along the visible violet. In this solar spectrum there is no perceptible effect in the more refrangible half of the violet, or in the ultra-violet region beyond.

The area most cleared of spores is not far from the Fraunhofer's line G.

Before discussing further the exact limits of action, and the region of maximum effect, I must say a few words about the method of marking the plates.

In the first place, it must be remembered that I cannot touch the film when once made, and covered with its quartz-plate: every possible precaution has to be taken that no foreign spores are allowed to invade and contaminate the agar, consequently the lines limiting the various coloured regions of the visible spectrum have to be drawn by hand, with a pencil, on the paper at the margins of the exposed slot-shaped area, and are therefore some millimetres distant from the surface of the film itself.

Secondly, it is, as is well known, far from easy to place a pencil point accurately on the limiting line dividing the green from the blue, and when we come to examine the spectra of the electric arc it will be seen that I have possibly shifted the line a little too much to the left in fig. 15.

The danger of this error is not obviated by the further procedure in marking these lines. When the plate is removed previous to incubation, I remove the coverings from the back glass—*i.e.*, the glass on which the transparent spore-laden agar-film lies—and mark as accurately as possible, with Indian ink, the lines corresponding to those drawn on the quartz-plate in front; this, of course, without shifting the relative positions of the two plates, and avoiding errors of parallax as much as I can.

Then I gum a thin strip of white paper on to the outside of this bottom glass plate, and continue the Indian ink lines on to it, and indicate with letters.

This done, the quartz-plate can be removed and a properly sterilised glass lid

* N.B.—These vertical lines are *not* the Fraunhofer's lines.

substituted, the Indian-ink removed from the glass, and the plate incubated. The marking of the negative taken from this is then done by super-position, and measuring off with compasses.

Fig. 14.

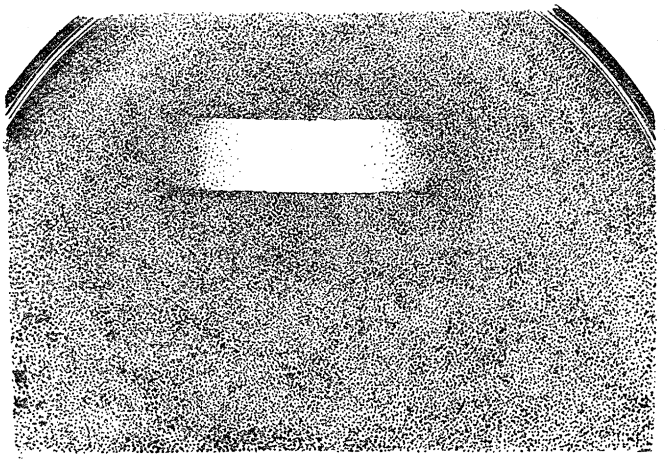
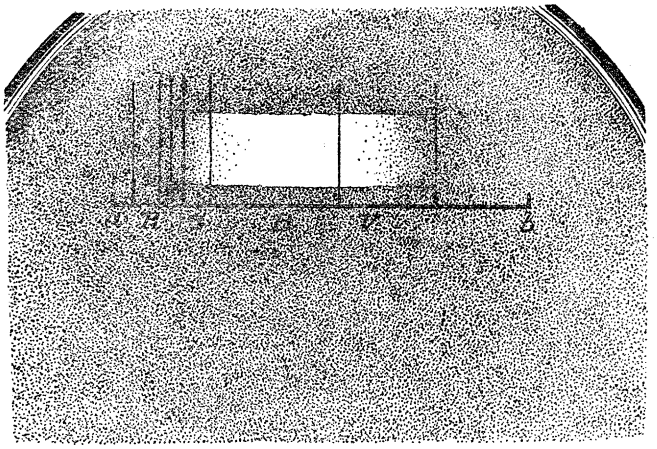


Fig. 15.



Agar plate of spores of *B. anthracis*, exposed to the action of the solar spectrum for 5 hours on August 16th (11 a.m. to 4 p.m.). The spectrum was very bright, and nearly, though not quite, pure. The photographs show the condition of the plate after 24 hours' incubation at 25° C. Fig. 14, simply the appearance of the film. Fig. 15, the same with the limits of the various regions of the visible spectrum marked on it (R the red, G the green, B the blue, V the violet). The base line, *a–b*, shows the length of the slot through which the spectrum shone on the plate.

All parts of the agar plate not exposed are rendered evenly opaque by the colonies germinated out from the spores. Similarly the spores in the infra-red, red, orange-yellow are unhurt, as are those to the other end of the violet.

The action of the light begins in the green-blue, and attains its maximum in the blue-violet, near the Fraunhofer's line G.

It must be confessed that there are several dangers of not getting these limiting lines quite accurate, but I have not been able to devise a way out of this difficulty.

But there is another source of possible error to be mentioned. I cannot do away with a certain degree of internal reflection from the inner surfaces of the glass and quartz-plates, and it is quite possible that a slight amount of over-lapping of the effect occurs at the boundaries. We shall see that with long exposures to the very intense electric-arc spectrum, this internal reflection becomes very marked indeed.

Finally, although I used as narrow a slit as possible, in working with the solar-spectrum I had to open it to at least 1 millim., and it is not improbable that this causes a slight shifting of the effect towards the red.

I am certainly of opinion that in fig. 14 the action appears to extend further into the green than is really the case, and that this is due to one or more of the causes referred to, and especially to the fact that the spectrum was not quite pure.

Experiments with the Light of the Electric-arc.

After a number of abortive attempts* to obtain results by exposing plates to the action of the spectrum obtained by decomposing the light from a powerful electric lamp, by passing the beam through a glass or carbon-bisulphide prism and ordinary lenses, I was fortunate enough to enlist the interest and co-operation of Professor Oliver Lodge, and it is to his cordial and sympathetic aid, in having my plates exposed to the spectrum in his laboratory, that I owe the remarkable success now attained.

We began by exposing different parts of an ordinary agar-plate culture of the spores of *Bacillus anthracis*, for different periods to the undecomposed light of the arc-lamp. The plates were prepared by me, exactly as if I were going to expose them to the solar light, as in my experiments described on p. 972. They were then sent off to Professor Lodge, who placed them at a distance of about two feet from a lamp whose nature and working were as described on p. 981.

We found at once that when the electric-light from such a lamp was employed, it required a much longer exposure for the rays, passing through glass, to act on the spores, than when the same plates were exposed to the solar light of a bright day, even in the winter or early spring.

Thus, an exposure of two to eight hours produced very little appreciable effect on plates which showed perfectly clear results after three hours' exposure to the sun on February 28; and even an exposure for ten to twelve hours to the electric light was far less effective than that to three hours' sunlight on the above date.

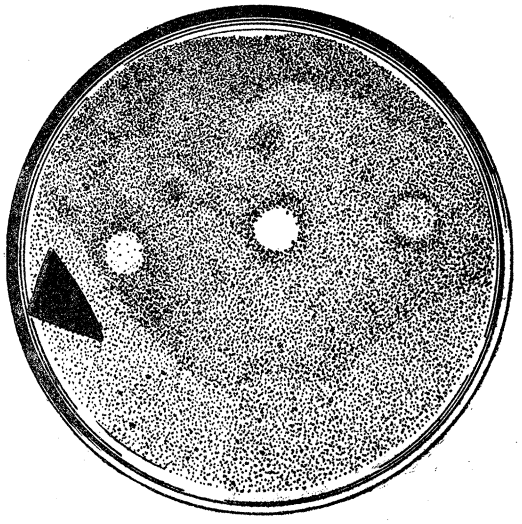
An exactly similar plate exposed to the arc-light from six to twelve hours, however, did give positive results in those parts to which the light had access for more than eight hours; but even where the illumination lasted for twelve hours, the effect was

* I have to thank Dr. Woodhead and Dr. Cartwright Wood, of the combined laboratories, for letting me try their lantern, and also my colleagues Professor Stocker and Mr. Shields, for exposing a plate for me; unfortunately the results of these attempts were negative.

only transient—by far the majority of the spores were only slightly retarded, or scarcely affected at all, for they germinated out later and covered the exposed areas as well as those not exposed.

In the above experiments the electric light traversed the relatively thick glass of the Petri's-dish. In the following experiment the film was protected by a thick plate of quartz over which a thin plate of glass was laid. Five circular openings were cut in the black paper covering the quartz, and one of these openings was covered up after 1 hour's exposure, a second after 2 hours, another after 3, another after 5, and the last after 8 hours' exposure. Nevertheless, even with the longest exposure the

Fig. 16.



Anthrax spores in agar exposed to the electric light, passing through glass. The centre circle shows the effect of 8 hours' exposure; that to the left, 5 hours'; that to the right, 3 hours'. Plate incubated nearly two days at 22° C.; on further incubation all the areas disappeared, as two have already done in the present case.

light-effect was very slight, and the numerous spores still alive rapidly covered the film thickly where exposed.

We, therefore, for the future employed quartz only, not only for the condensers of the lantern, but also to cover the films, and have since always done so—in short, we found that even the thinnest plate of glass is so obstinate a barrier to the bactericidal rays that it is not permissible to use it.*

This is readily seen from the results of succeeding experiments.

The plates themselves, also, had to be so prepared that, instead of the light traversing the glass of the Petri-dish, it must traverse a quartz-plate placed over the film. This was arranged as follows.

* That the highly refrangible rays of the electric arc are absorbed by glass was shown by Sir Gabriel Stokes, in 1853.

The plate is prepared as usual, by the melted agar (B, fig. 17), infected with spores, being poured, at 50° C., and allowed to set as a thin transparent film on the bottom of the sterilized glass Petri-dish, A.

When properly set and cool, the glass lid of the Petri-dish is removed and replaced by a plate of clear quartz (C), cut flat and polished, and, of course, sterilized by heat. This is held in position by sterilized gummed slips. Then the whole is wrapped closely in sterilized dead black paper, D, followed by tinfoil, F, and an outer wrapping of white cartridge paper, G.

Then the suitable windows are cut through the three wrappings, as shown in section at E, and the plate is ready for exposure.

To ensure success with these plates several precautions are necessary in order to guard against certain dangers. Some of these precautions are those needed for all such plates, others are peculiar to the circumstances of this special case.

Fig. 17.

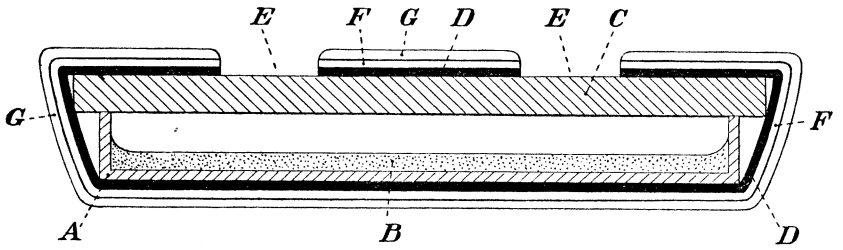


Plate culture of spores of *Bacillus anthracis*, devised for exposure to the light of the electric arc. A is the glass Petri-dish, in which the agar film B is confined. C is a plate of clear quartz, so arranged as to serve as a lid to the glass dish. DD, dead black paper, enveloping the whole, except where cut through, at EE, which are openings exposing the quartz-plate (and the underlying film) to the light. Over the black paper comes tinfoil, FF, and a wrapper of thick white paper, GG.

In the first place, good sharp results can only be obtained if the spores are vigorous and properly distributed in the agar film. If too many, some spores shade the others from the action of the rays passing through the windows, and a faint figure only is obtained, and rapidly obliterated by the subsequent germination of the protected spores; if too few, the contrast between the exposed and unexposed areas is not sharp enough.

Secondly, the agar film, on cooling, is apt to exude so much water that the condensed fluid accumulating between the glass and the film causes the separation and *slipping* of the latter as soon as the plate is tilted. I found the best way of preventing this is to pour the agar, at about 50° C., into the glass dish when the temperature of the latter is about 40° C., having cooled to that from a high temperature, and so to arrange matters that the lid of the dish—or the quartz-plate—should be about 10° to 15° C. cooler than the bottom, and then to allow the whole to cool to the ordinary temperature *very slowly*. This ensures that the glass bottom is perfectly dry, and that the condensation of the moisture from the cooling film shall

occur chiefly on the lid. In some cases it was even necessary to put sterile filter-paper between the lid and the dish, to absorb the large quantities of moisture. I also found it advisable to employ agar tubes kept some time, until the greater part of the water had evaporated from them. By these means the agar-film remains in contact with the glass, and does not slip afterwards.

Thirdly, a special objection takes its source in the necessarily appreciable space between the surface of the agar-film and the inner surface of the quartz-plate. For I found that unless the rays of light fall perfectly vertical on the agar-film, we are apt to get blurred images; and even when they are nearly vertical, so much reflection of the rays results from their striking, first the glass bottom of the dish (through the transparent agar), and then the black-backed polished quartz-plate, that secondary effects are produced traceable to the action of these internally reflected rays.

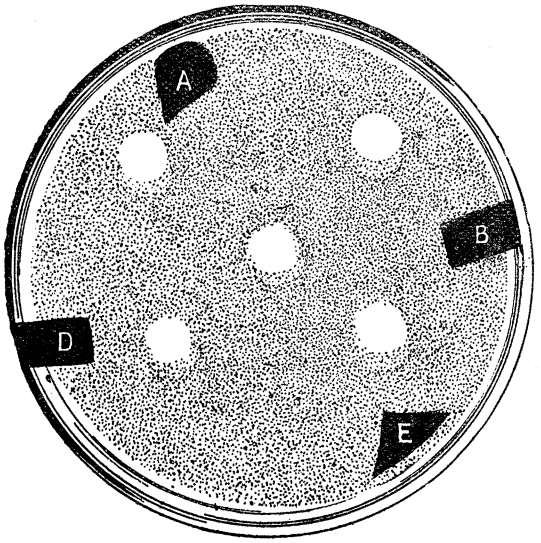
In most cases I reduced this danger to a minimum by using dishes as shallow as possible; but in special instances it was necessary to use sterilised dead black paper on the internal face of the quartz. The principal objection to this latter device, however, consists in the danger of the moisture of condensation so damping this black paper as to render it limp and loose, and make it fall down on to the agar.

These, then, are some of the principal troubles, and causes of failures which had to be contended with. But there are others, and when one reflects how difficult it is to successfully overcome all the obstacles simultaneously in the case of any particular plate, it can hardly be matter of surprise that these experiments have consumed much time, and often resulted in disappointing failures.

One of the worst troubles was the tendency of the spores to germinate *en route*, under the influence of the rising temperature as the weather got warmer, or in the laboratory in Liverpool. This had to be met by icing the plates, packing them in ice, and keeping them on a metal ice-box during exposure. But this led to new difficulties, for since the plate must be packed off so as to be exposed as soon as possible after making, the rapid cooling of the film leads to rapid and copious contraction and condensation of exuded moisture, thus exaggerating the very dangers I have been discussing. Over and over again I have had the plates returned in such a damp condition that the film has slipped, spores have been washed from the unexposed parts on to the exposed ones, and a troublesome and careful experiment, which has occupied much time on the part both of Professor LODGE and myself, came to nothing. Not the least of the great obligations I am under to Professor LODGE is due to his patience and willingness to repeat such experiments for me.

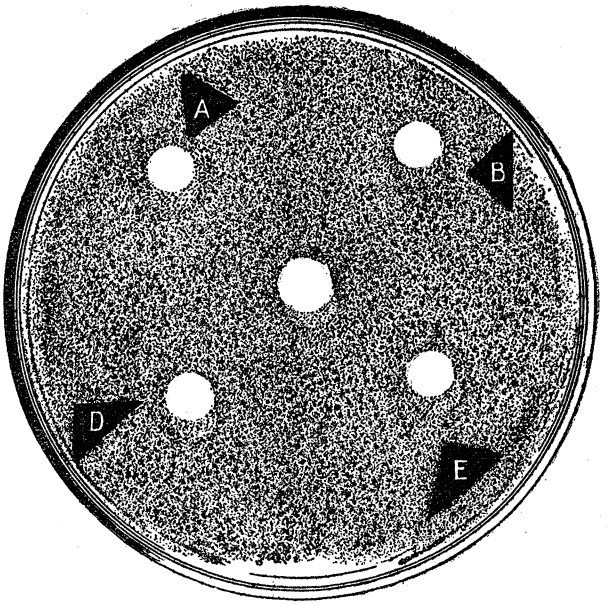
A plate, fig. 18, prepared as above, and with five circular windows, was exposed to the undecomposed light from the arc lamp, on March 21, so that the windows, marked A to E, were blocked out with tin foil, as follows :—A, after 3 hours' exposure; B, after 4 hours'; C, after 6 hours'; D, after 5 hours', and E, after 2 hours'. The plate arrived back on March 22, and was put into the incubator at 2 P.M. on that date. The temperature of incubation was 25° C.

Fig. 18.



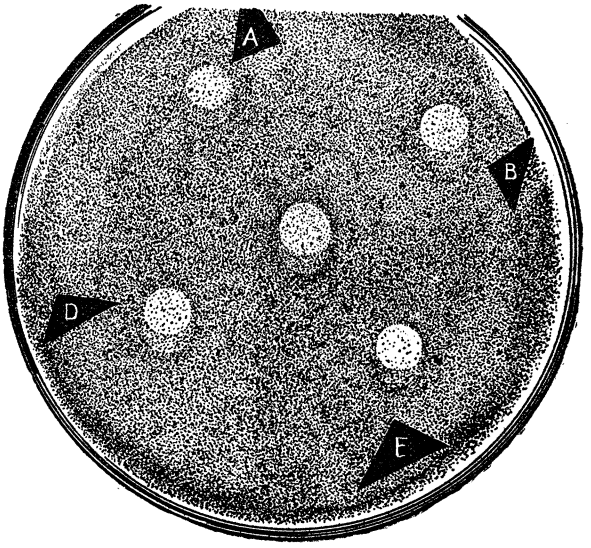
19 hours' incubation.

Fig. 19.



48 hours' incubation.

Fig. 20.



77 hours' incubation.

Figs. 18–20. Agar plates of anthrax spores, covered with quartz, on which five circular windows were exposed to the arc light on March 21. The windows are A, to the left above; B, to the right above; C the central one; D, to the left below; E, to the right below.

A = 3 hours' exposure.
B = 4 „ „
C = 6 „ „
D = 5 „ „
E = 2 „ „

The culture-plate was photographed from the front, *i.e.*, as exposed, with black paper behind. All five circles are just visible, after 19 hours' incubation, at 25° C.

At 9 A.M., on March 23, the plate was photographed, with the result shown, fig. 18. A very faint* circular image of each window was becoming visible, and in two hours later differences could be detected between the various circles as regards distinctness After a further day's incubation the five circular areas were each perfectly sharp, as shown in fig. 19, and differences were still more observable. After yet another day the plate was at its best, or, strictly speaking, a little past its best, as seen in fig. 20.

On comparing the figures we notice that the relative sharpness depends on how far each circular area is cleared of spores capable of germinating. As a matter of fact all five areas on the third day had some spores still living on them, but it was evident that the two top ones, A and B, had fewer than the two bottom ones, D and E, and even than the centre one C—facts brought out more clearly in the photographs than in the reproductions.

If each area had an equal number of spores on it to begin with, we should expect the order of clearness, *i.e.*, the sharpness of the figure, to be in the order of length of exposure. C was exposed 6 hours, D only 5 hours, whence C should be clearer than D. Now in the figure this is not the case. But it *was* the case the day before, and the only reason that C is not *now* the clearer is because a large number of spores have germinated on the area C, and are rendering it somewhat obscure.

As a matter of fact, the order of clearness of the areas was just what we should expect in Stage III.

The "ghosts" or faint secondary circles on this plate are not easy to explain. I thought they were due to internal reflection, but it is not improbable that they were due to the plate having received a jolt during an advanced stage of exposure, and the angle of the incident rays to the surface of the agar having been thereby altered.

This experiment shows, then, that 6 hours' exposure to the rays of the electric arc, transmitted *viâ* quartz, results in the death of an enormous number of spores, and it is very probable that only such spores escaped on D and C as were directly behind (and therefore screened by) a number of others. The film was a dense one, and such contingencies were certain to arise. It may be regarded as extremely probable, that had every spore been equally exposed to the rays, an exposure of 6 hours would have been quite sufficient to kill every one.

It may be regarded as demonstrated, therefore, that exposure to the light of a powerful electric arc, provided the rays are not kept back by a glass or other screen opaque to the more refrangible rays, results in effects similar to those obtained by exposure to strong sunshine; and I venture to throw out the suggestion that one of the most effective methods of disinfection of hospital wards, cattle sheds, railway trucks, &c., may be found to be practicable along these lines. This, too, be it observed, is quite independent of our knowledge of the *modus operandi* of the bactericidal rays on the spores or bacilli. I mean, it is immaterial in practice whether the light rays act on the contents of the spores, &c., or by liberating some volatile

* Too sharp in the reproduction.

body which diffuses into and poisons the protoplasm, since my experiments with dried films of spores show that so long as the right rays reach the spores they are killed by a few hours' illumination. I have already given the evidence which leads to the conclusion that the bactericidal action is *direct* on the spore contents, moreover, and only put the above proposition in that form to meet any possible objection on the part of those who refuse to regard that evidence as conclusive. However, I shall revert to this matter when discussing the question of the action of the various rays of the spectrum, to which I now turn.

We now proceeded to expose plates, prepared substantially as above, to the rays of the arc-lamp, decomposed by means of a quartz prism so as to form a spectrum.

After several attempts, and some disheartening failures, due to one or other of the causes already referred to, the following arrangement was resorted to:—The Petri-dish, with its film, as in fig. 17, was covered with a thin glass plate, in which one or two slots, about half an inch wide and two and a half to three inches long, were cut through. Then the quartz plate was put over this, with black paper, pierced in accordance with the slots, between. Everything else was as before.

The lamp and quartz train were arranged as follows:—

Fig. 21.

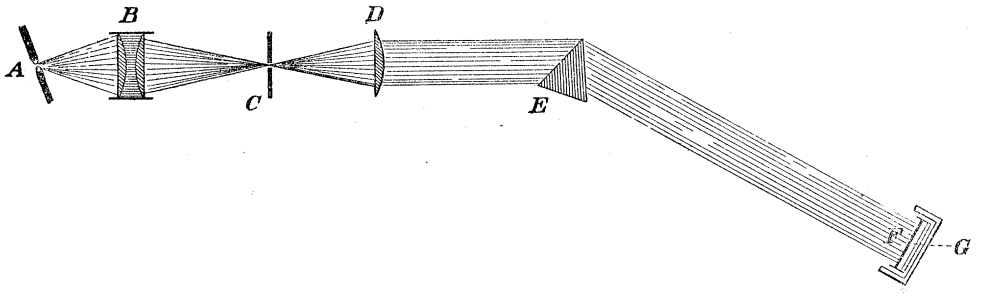


Diagram of the arrangement for directing the spectrum of the electric arc on the prepared film of agar with spores or bacilli in it.

A = the electric arc light, passing through the quartz condensing lenses ($4\frac{1}{2}$ in. diam.) at B. C, the slit. D, quartz focussing lens, which directs the beam on to the quartz prism, E. F, the spectrum, falling vertically on the quartz plate of the Petri-dish, G, which lies in an iced box. Distance from A to $B = 4\frac{3}{4}$ in.; from B to $C = 7\frac{1}{2}$ in.; from C to $D = 6\frac{1}{2}$ in.; from D to $E = 9$ in.; and from E to $F = 21\frac{1}{2}$ in.

I now proceed to the analysis of one of the most satisfactory and instructive spectra of the electric light yet obtained.

The plate was made on August 2, and exposed for 12 hours to a very pure spectrum on August 3, 1893. The film of anthrax-spores in agar, was covered by a glass-plate with two slots in it, and dead black paper over all parts of this glass except the pierced slots. Over these slots a thin quartz-plate was secured, and, before exposure, a very thin sheet of good glass was placed over the upper slot. Consequently the

spectrum had to traverse a thin sheet of glass before reaching that part of the agar-film which lay under the upper slot, while that part under the lower slot received the spectral rays through thin quartz only.

The exposed plate was placed in the incubator at 22° C., at 6 P.M. on August 4, and was incubated in all four complete days: it was removed at intervals to be photographed, the camera being so arranged that the image falling on the photographic plate was exactly the size of the original. The incubated plate to be photographed was so placed that daylight from a north window, after traversing a screen of oiled white tissue-paper, passed through it from back to front; consequently the photograph represents the view from the front of the plate, as one would see it on looking into the Petri-dish. The quartz plate was removed for photographing, after a standard copy of the lines dividing the various colours of the spectrum had been made and fitted to the plate.

After 40 hours incubation, *i.e.*, at 10 A.M. on August 6, this plate showed two very distinct, though, as yet, faint, clearer areas, corresponding to those parts of the film which lay beneath the two slots, as shown in fig. 1, Plate 87.

In fig. 2, Plate 87, I have shown by means of vertical lines the subdivisions of these areas into the various parts of the spectrum.

In each case the exact length of the slot, and therefore of the portion beneath the visible and invisible parts of the spectrum, is marked by the distance A–B.

The line C indicates the beginning of the visible red of the spectrum: consequently the area to the left is the infra-red. Between D and E are the orange and yellow regions. E to F includes the green; F to G is the blue; and G to H the visible violet. To the right of H is the ultra-violet, of course invisible.

Several facts are obvious at a glance. In the first place the bactericidal effect begins almost exactly on the boundary between the green and the blue, *i.e.*, at the line F in the photograph,* and, in the lower spectrum, extends right away into the ultra-violet. In the upper spectrum this extension of the effect into the ultra-violet is much curtailed, owing to the opacity of the glass for these rays.

Secondly, the maximum effect, as seen by the widening out of the affected region, is just beyond the violet. I have already pointed out that I explain this widening as due to the action of the most potent reflected rays being still powerful enough to produce some action.

Thirdly, it is clear from the first that the infra-red, red, orange-yellow, and green rays are without perceptible effect.

These facts became only the more certain as the incubation proceeded. Fig. 3, Plate 87, shows the plate after 64 hours, and fig. 4, after 88 hours. It is observed that the bactericidal action at the margins in the region of maximum action is much more partial, and the spectrum consequently takes on more and more the shape of the slot.

* It will, of course be noticed that these are not the FRAUNHOFER'S lines, but the boundaries between the visible and other regions.

The two or three boss-like protuberances on the lower margin of the lower spectrum, situated in the ultra-violet, are expressions of the shading action of some drops of Canada-balsam—used for cementing the quartz-plate over the slots, and browned by heat during sterilization—which projected beyond the edge of the slot in the glass plate. They are particularly evident in fig. 4, and there also we see that the bactericidal action is not complete at the right hand end of the ultra-violet; though, had the slot been longer, it is pretty evident that the partial action would have extended further. Nor is the action complete at the line F, for we see many spores are now germinating there.

Even in the most perfectly clear areas, also, there are still a few spores alive, as is yet more evident in fig. 5 (112 hours), and fig. 6 (136 hours). This, I explain, as due to an odd spore here and there being so completely sheltered behind others, that the rays have not reached them, or only to a feeble extent, and consequently they germinate out later, and develop isolated colonies. This is particularly well seen in fig. 6. There is nothing surprising in this latter fact when we reflect how many millions of spores were present over the illuminated area; and it is at the same time an excellent reply to those who would regard the agar as in any way rendered an unsuitable pabulum for the spores to germinate on, by the chemical action of the light on it.

The action of light on other Bacteria, Yeasts, Fungi, &c.

That light, as a whole, exerts a similar destructive effect on bacteria other than *B. anthracis*, follows from the researches of most of the previous observers,* from Downes and Blunt onwards; but, with the exception of Buchner,† their methods are open to all the criticisms already given. Nevertheless, putting aside all attempts at *quantitative* results, and overlooking their contradictory statements as regards what rays are active, we cannot avoid the conclusion that light exerts some destructive action on Typhoid (Janowsky and Buchner), *B. coli-commune* (Buchner), and several non-pathogenic forms of Bacillus and Micrococcus (Duclaux, Gaillard, Buchner, Kotljar, and others).

Contradictory statements occur, however—*e.g.*, Lubbert‡ says light has no action on *Staphylococcus pyogenes aureus*, whereas Chmelewsky§ concludes that it has.

Elfving‖ and Laurent¶ have shown that light has, at any rate, *some* action on

* See my previous papers and the Reports to the Water-Research Committee for the earlier literature.

† *Op. cit.*, 1892.

‡ 'Der *Staphylococcus pyogenes aureus* und der *Osteomyeletes micrococcus*,' Würtzburg, 1886, p. 14.

§ 'Centr. f. Bakt.,' 1892, vol. 12, p. 174.

‖ 'Studien ü. d. Einwirkung des Lichtes auf die Pilze,' 1890.

¶ 'Ann. de l'Inst. Pasteur,' 1888.

moulds, and I have given a *résumé* of the other references in the literature elsewhere,* tending to the same end, as well as of my own experiments.

I now add the results of some experiments which go to show that this injurious action of light is probably common to all the lower forms of vegetable life whatever, and even to all living protoplasm, although, as I showed in the paper referred to, the action may be warded off in ordinary cases in nature by means of natural coloured screens.

PRINGSHEIM'S experiments on algal cells† may, I think, be taken in evidence to the same end, though it has been the custom to somewhat overlook their importance, partly on account of his extreme hypothetical ideas, and partly because he used such intense light focussed on the cells.

The investigations of botanists, showing the inhibitory action of light on growth, will also come under the same head, and, judging from the little I have learnt of the action of the blue-violet rays on animal cells,‡ there seems reason to conclude that the action is universal on living protoplasm.

Mr. NORMAN LOCKYER has told me of the damaging effect—expressed in symptoms reminding one of snow-blindness and sun-burn—of working with the unguarded arc-light, and it is not improbable that much more evidence to the same end could be gathered.§

The degree of injury will, of course, be different in different cases, and the injurious action of the blue-violet rays in specific cases is by no means contradictory of instances where their general effect is for good.‖

The following experiments were made with a remarkable violet bacillus from the Thames, the deep violet pigment of which is rapidly destroyed by light, though its solution withstands evaporation to dryness over a water-bath without any trace of injury.

On March 31, a bright clear day, the agar-plate was exposed for two hours—12 noon to 2 P.M.—with no screen and over a mirror. A stencil N was placed over the only exposed part of the plate.

The accompanying figure shows the condition of the plate at 9 A.M., on April 2—*i.e.*, after 43 hours' incubation at 20° C., showing clearly that this bacillus is very sensitive to sunlight.

* 'Roy. Soc. Proc.,' vol. 53, 1893, pp. 37, 38.

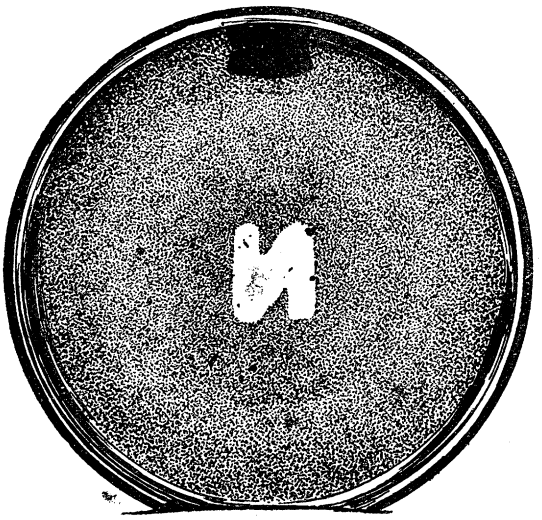
† 'Jahrb. f. Wiss. Bot.,' vol. 12, 1881.

‡ See RAUM, 'Zeitschr. f. Hygiene,' vol. 6, 1889, pp. 312–368. SIEMENS ('Roy. Soc. Proc.,' 1879) may also be consulted in connection with this subject.

§ At a recent lecture before the Camera Club, a member kindly called my attention to an experience in a German electric light works, where the medical men traced the irritation suffered by the workers to the action of the violet rays from the arc lamps.

‖ As expressed in the Italian proverb, to which Mr. GALTON first drew my attention, *Dove non va il sole va il medico.* On the subject of the sun baths employed on some parts of the Continent, and to which my attention has been kindly directed by a member of the Camera Club, I cannot give any opinion.

Fig. 22.



Similar results, but showing differences which suggest differences in sensitiveness—though I reserve any definite expression of opinion as to what that means at present—have been obtained with several other Thames bacteria, *e.g.*, *B. fluorescens liquefaciens*, a pink Bacterium (probably *B. prodigiosus*), and some others, and with the hay Bacillus, the potato Bacillus, and others.

The following illustrates the action on a yeast. The form used was *Saccharomyces pyriforme*, the yeast of the "ginger-beer plant." It was exposed for about 8 hours in all to reflected light, not very bright, on February 7th and 8th. An excellent photograph was obtained, showing the condition of the plate after 48 hours' incubation at 20° C.

Similar results have been got with other yeasts, and with *oidium*. The chief difficulty with these *oidia* and fungi is to get the spores properly distributed in the films, and, since their spores and mycelia are so large and relatively coarse, the photographs are not so sharp and clear as are those got with bacteria.*

But I have also results with screens and with the spectrum, which show that the generalization as to light-action being universal on all lower organisms (and no doubt all living cells) is not unwarranted.

Other photographs show the effect of the solar spectrum on a plate of the violet Bacillus already referred to. It was exposed for 2 hours on August 25th, and again for 2 hours on August 26th, neither of them very good days for the work; and still the spectrum had very decided effects. The plate was incubated 24 hours.

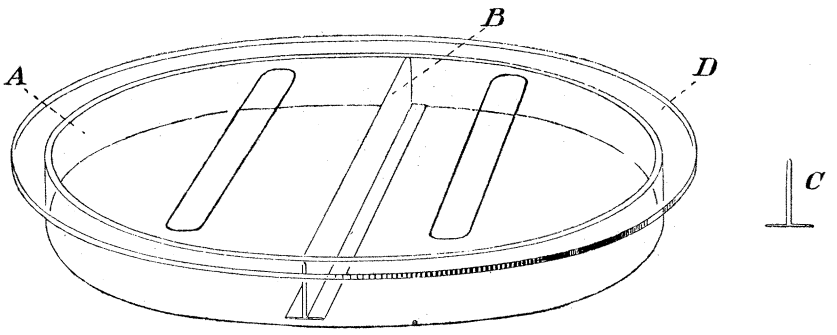
Similar spectra have been got with *B. subtilis*, *B. fluorescens*, *B. prodigiosus*, and some others. I reserve the discussion of their peculiarities for the present, merely remarking that they agree generally with what has been said.

* These results with yeasts and fungi are interesting in connection with a recent publication by Martinaud ('Comptes Rend.,' CXIII., No. 22, p. 782), who finds that direct exposure of grapes to the sun kills the two common fermentation yeasts normally met with on their surfaces.

I am at present concerned with an attempt to photograph the electric spectrum simultaneously on *two* species of bacteria on the *same* plate. Unfortunately there is delay, owing to an accident to the electric instalment, kindly put at my disposal, as said, by Professor LODGE; and it seems better to defer the discussion of what results are already to hand.

For the sake of any who may be inclined to carry on this work, however, I append the following description of the plates I am employing:—

Fig. 23.



A is an ordinary Petri-dish of glass. B is a partition of talc, so arranged that it is partially split into two flanges, shown in section at C. I cement the flanges to the glass bottom of the dish with Canada balsam, and sterilize the whole. Then I pour equal agar-films, each charged with a different organism, into each of the two halves of the plate. The plate is then covered with a glass plate, D, in which two slots are cut, each slot so arranged that it lies over the middle of each half-film. The quartz plate then goes over the slot, and the sterilization wrapping and exposure, &c, are made as described on p. 977.

There are difficulties in manipulation, but I find they can be overcome; and even already the results promise to be worth the trouble. Of course the main object is to compare the effects of the spectra on the two organisms under like conditions.

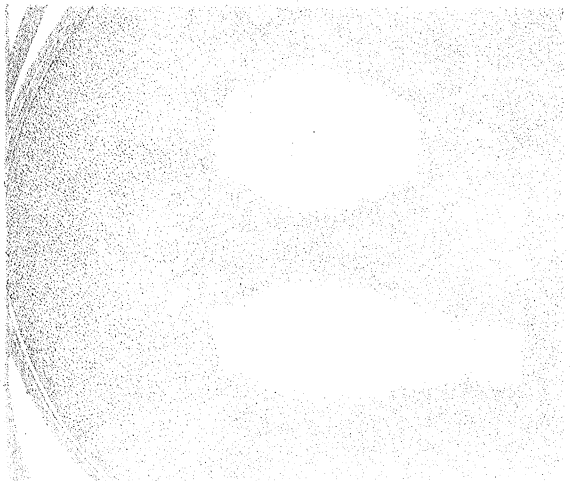


Fig. 1.

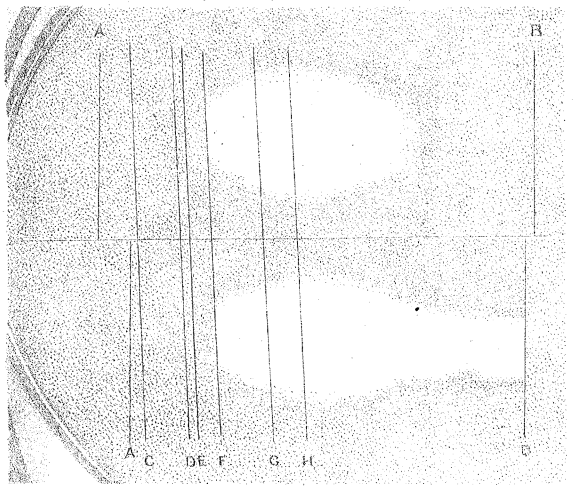


Fig. 2.

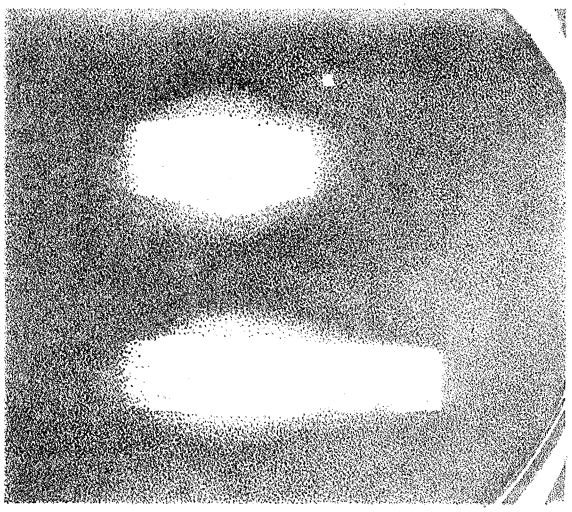


Fig. 3.

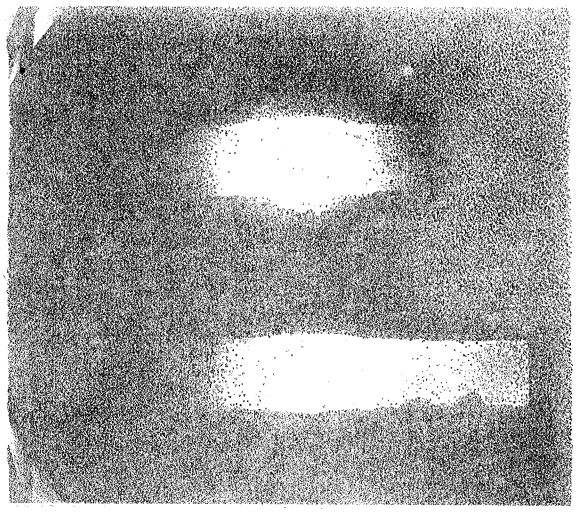


Fig. 4.

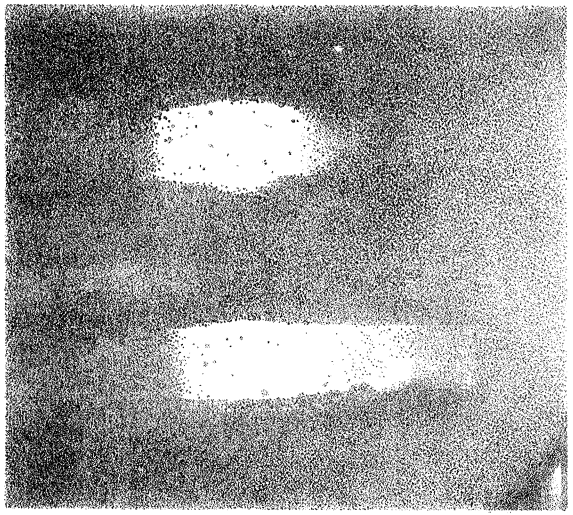


Fig. 5.

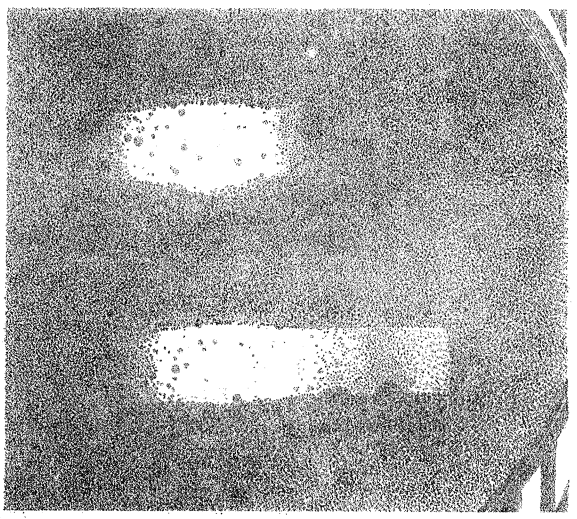


Fig. 6.

West, Newman lith.

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

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Fig. 1.

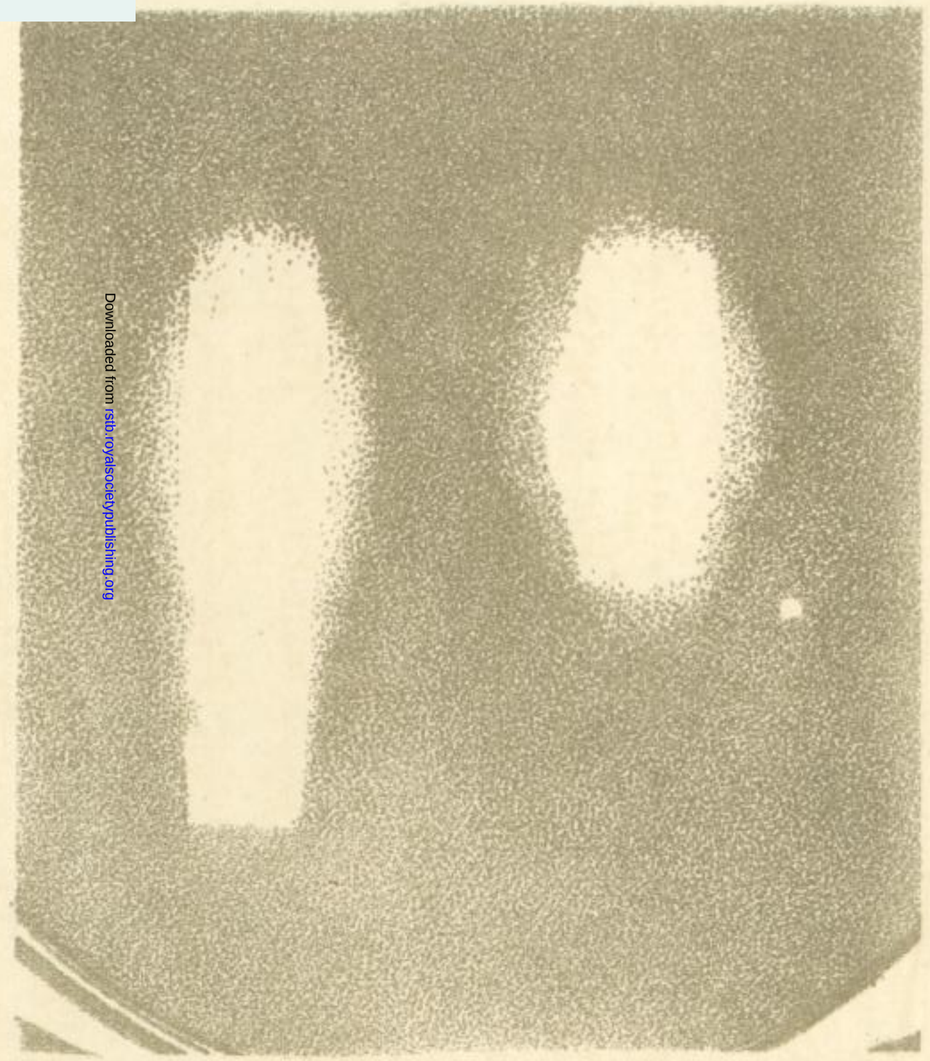

Fig. 3.

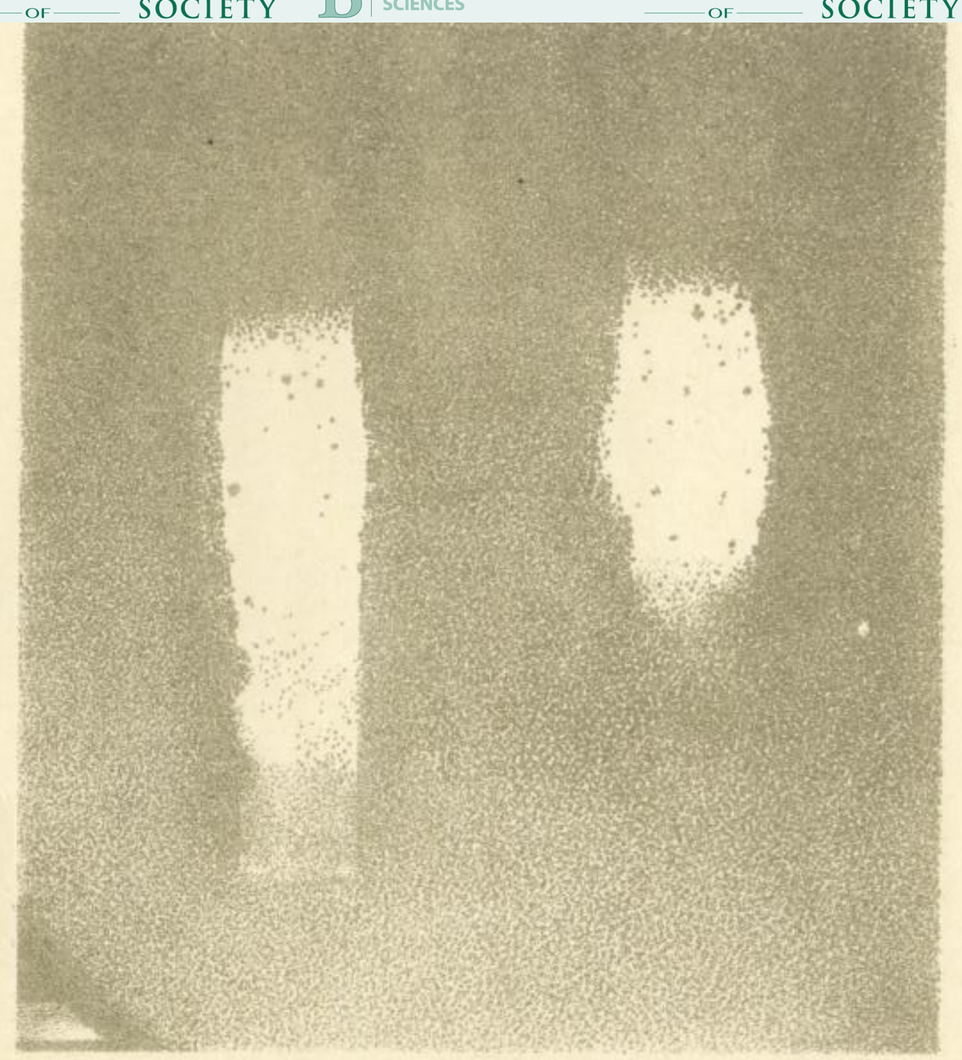

Fig. 5.

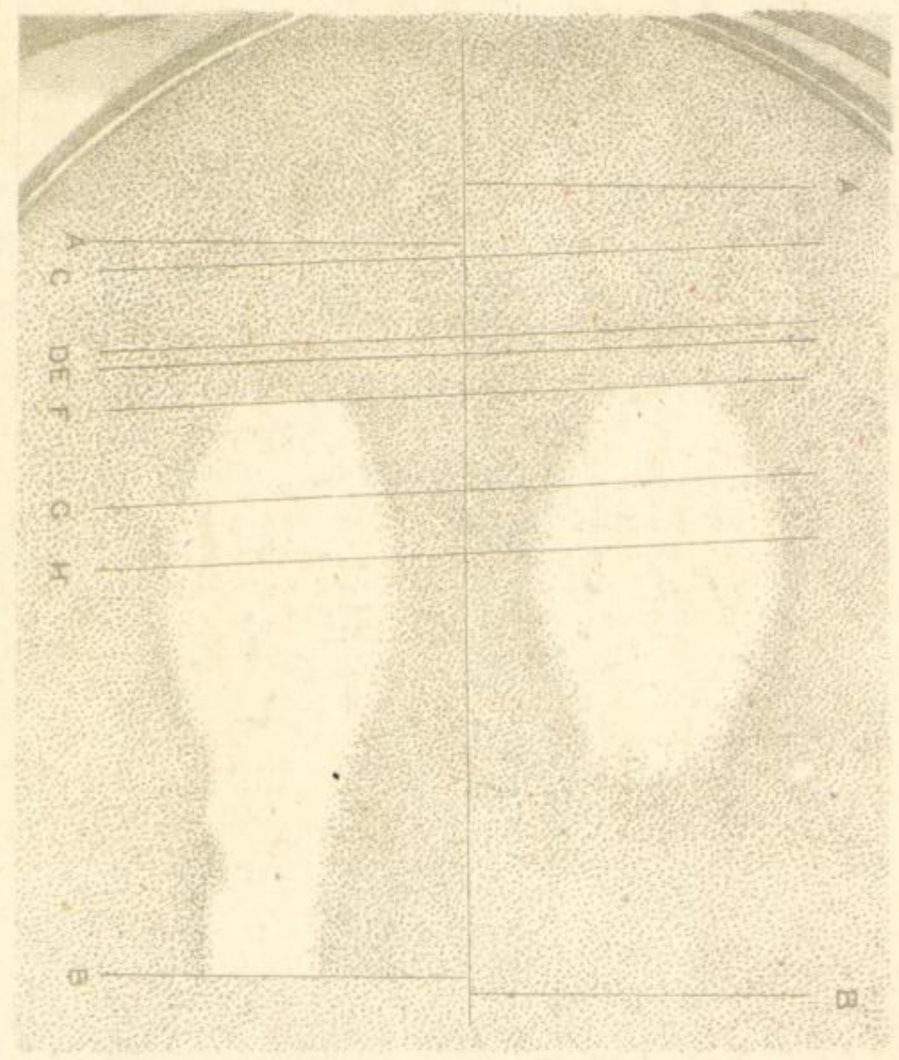

Fig. 2.

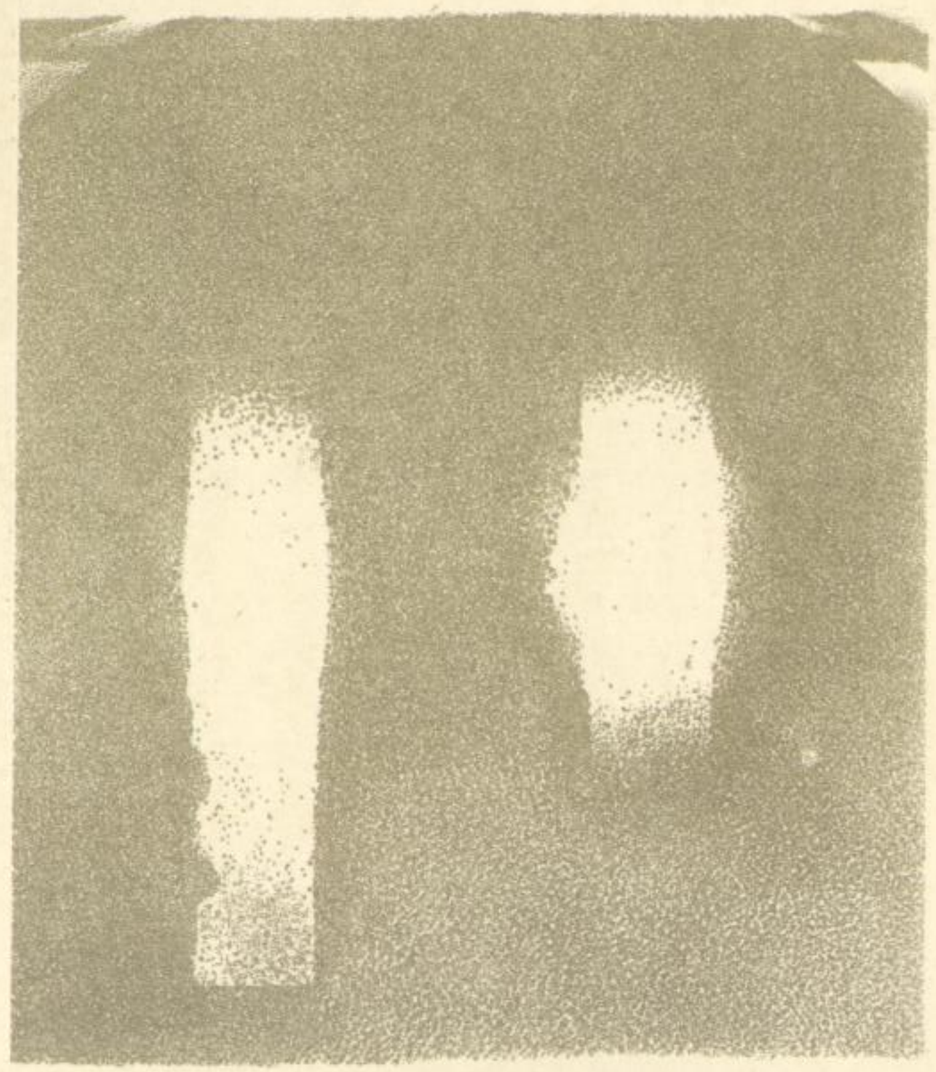

Fig. 4.

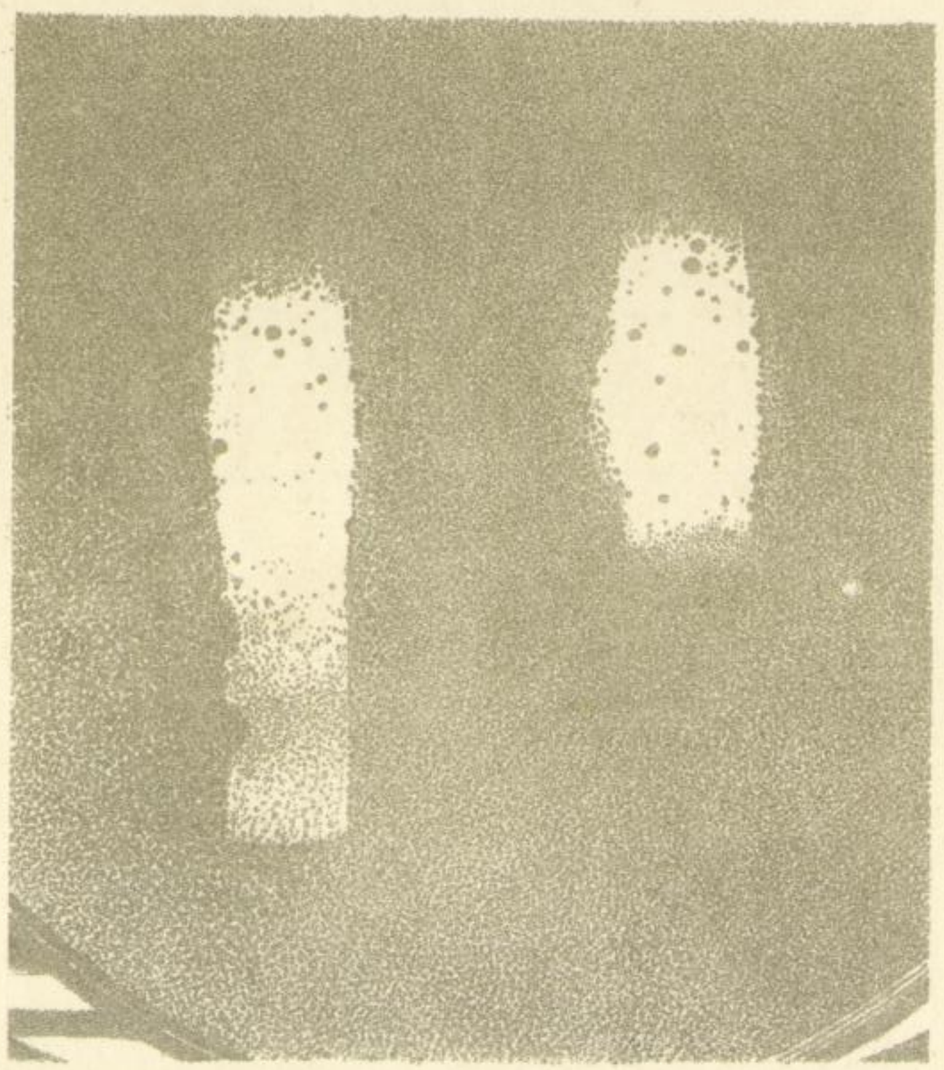

Fig. 6.