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(WITH PLATES 2 AND 3)

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INTRODUCTION.

THE action of sunlight upon living bodies has always been a problem full of interest to the biologist, and even to the chemist and the physician. The well-known effect upon green plants in which carbon dioxide from the air and water from the soil are gradually changed, through the influence of sunlight, into starch, cellulose, and sugar, naturally led many to believe that sunlight exerted an important influence upon other life as well. With respect to the animal species we know comparatively little, only the lower members, as the protozoa, having been extensively studied; on the other hand, the lower plant forms, especially the colorless plants, early received systematic investigation. The bacteria received the most attention, undoubtedly because of their relation to disease and to national and household economy. The new methods of work which bacteriology originated promoted this sort of investigation; as a result, we possess today an extended literature upon the question of the action of light upon the bacteria. This action is described as bactericidal, the non-spore-

forming species being killed in about one and one-half hours in summer, and two and one-half hours in winter.

One phase of this subject appears, however, to have received little or practically no attention; this is the effect that light exerts upon the bacillus of tuberculosis. When it is recalled that tuberculosis carries off one-seventh of the human race, not to mention the accompanying suffering and financial sacrifices that its long-drawn-out character entails, it appears that this organism should have received early and abundant attention. The cause of this neglect is not difficult to understand. In the first place, the tubercle bacillus is a slow grower when compared with most other bacteria. A day or two will give satisfactory growths with many, while the tubercle bacillus requires two to four weeks. In the second place, tubercle bacilli will not grow on the ordinary media, but require proteids such as coagulated blood serum or egg albumen. The temperature, too, must be held rather closely to 37° C., and the moisture must be carefully conserved. Taken together, these facts have evidently deterred workers in this field from employing the tubercle bacillus.

The question of the action of sunlight upon *B. tuberculosis* was taken up by the writer in the summer of 1905. It was soon found, however, that the conventional methods for testing the action of sunlight upon bacteria were not feasible in working with *B. tuberculosis;* and, indeed, this is another reason why so little work has been done upon this important organism. In attempting to determine a suitable method, it was found that the slow-growing character of the culture made it unsuitable for the rapid testing of new methods; other bacteria were employed for this purpose, *B. coli* answering the purpose admirably because of its vigorous growth, its heavy cloudiness in bouillon, its resistance to desiccation, and, more especially, the readiness with which it could be identified when occasion demanded. Consequently this organism has been used in a large part of the work, and the report made to include these tests. As will be seen presently, this appeared most desirable, for the application of new methods required a comparative study, and this study at once showed certain defects in all the data that had heretofore been gathered. Before proceeding farther, we may with advantage review these data briefly; not that they are few, for they fill many pages, and a simple bibliog-

raphy would include perhaps a hundred titles; nor, indeed, because they are unimportant, for they have served as necessary stepping-stones in our progress.

For bibliographies, the reader is referred to the publications of Marshall Ward, Dieudonné, and Raum. Here only the epoch-making researches, and such as bear directly upon the present line of work, need be considered. These will be presented in some detail in order that direct comparisons may be made with the work presently to be recorded.

HISTORICAL REVIEW.

A. *Action of light on bacteria in general.*—The fact that light exerts a detrimental influence upon the colorless plants must have been recognized at an early date; it was most natural, therefore, when bacteriological methods were sufficiently developed, that considerable attention should have been paid to this group. Within a decade after Koch's discovery of the solid media, many of the important species were tested as to the action of sunlight upon them, and the results recorded. The past decade has added little that is new to this field, save the testing of X- and Radium rays.

The earliest work, preceding even the days of solid media, is that of the now classical investigations of Downes and Blunt.[1] They were, indeed, the first workers in this field. They exposed broth cultures containing a mixture of organisms, and observed a decided inhibitive action. Days or even months were required to sterilize the cultures.

Another early worker in this field was Arloing.[2] He used cultures of anthrax, and came to the conclusion that if spores were present they were not killed out until 25 to 30 hours had elapsed. In an earlier trial, broth tubes containing spores, which had been exposed for two hours in July, remained sterile. If allowed to germinate, 25 to 30 hours were required to kill.

Using one of his Tyrothrix forms which produced spores, Duclaux[3] found that in bouillon tubes:

after	5 days'	exposure	1	out of	3	remained	sterile
"	1 month's	"	2	" "	3	"	"
"	2 months'	"	3	" "	3	"	"

When micrococci were employed[4] the time required to kill them was decidedly less, not more than 40 days in the spring time, or 20 days in summer. If exposed in a dry state, eight days in spring and two to three days in summer sufficed to kill.

Marshall Ward[5] also worked with anthrax, but substituted agar plates for bouillon tubes. Gelatin was tried, but this medium melted in the sun, and so gave uncertain results. His agar plates were covered by a black stencil. The results were not very definite, and varied with the brightness of the days. In March, exposures of one-half

[1] *Proc. Roy. Soc. Lond.*, 1877, 26, p. 488; and 1878, 27, p. 199.
[2] *Comp. rend. de la Acad. Sci. Paris*, 1885, 100, p. 378, and 101, pp. 511, 535.
[3] *Ibid.*, 100, p. 119.
[4] *Ibid.*, 101, p. 395.
[5] *Proc. Roy. Soc. Lond.*, 1890–94.

to one hour inhibited the growth; and one to one and one-half hours killed. He tested the action of the various colored rays, with the general result that the more refrangible (green to violet) rays showed germicidal powers upon both spores and bacilli.

Working about the same time as Ward, we find Buchner,[1] whose classical demonstration of the sun's bactericidal action on a typhoid agar plate is so well known as scarcely to require mention. The plate was covered with black paper through which the letters of the word "typhus" were cut; this plate was exposed to direct sunlight and then developed in the dark. In the letter openings the bacteria were killed, and when the paper was removed, the letters stood out boldly.

In another experiment he made an aqueous suspension of *B. coli* containing 100,000 germs per c.c. After exposing to direct sunlight for one hour, the suspension was found to be sterile.

Dieudonné[2] experimented with *B. prodigiosus*, and *B. fluorescens putidus*, especially because they were supposed to be highly sensitive to light-action. He found that in agar and gelatin plates, one and one-half hours of exposure kill the bacteria in March, July, and August; while two and one-half hours were required in November. Repeating the work with *B. coli*, *B. typhosus*, and *B. anthracis*, substantially the same results were obtained. He also investigated the effect of the different rays by the use of colored solution screens, and by the prism with an arc light. Both confirmed Ward's work. As to the question whether the action was primarily upon the medium and so indirectly upon the bacterium, or upon the organism direct, he believed the latter was mainly the case, although the medium was somewhat changed.

Kruse[3] worked more especially upon secondary questions, such as the effect of the different colored rays, using colored solutions for his screens; also the influence exerted by the presence of gases, such as hydrogen and oxygen. In oxygen the bacteria, when exposed to sunlight, were killed in three hours, while in hydrogen they were still living after six or seven hours. He also tested the action upon the culture medium; while this was affected, the growth being somewhat inhibited, still this was not sufficient to explain the action of sunlight upon bacteria.

Recently some experiments were made at Lawrence, Mass., by Clark and Gage.[4] The typhoid and colon bacilli were employed, watery suspensions of fresh cultures were made, and 1 c.c. of the suspension was placed in a petri dish which was then exposed to direct sunlight. After exposing a definite time, agar was added to the water and the plates incubated. In 10 to 15 minutes' exposure, 95 to 99 per cent of the typhoid germs were killed, and 96 per cent of the colon germs. To destroy completely all life, the time varied from one-half hour up to four hours. These tests were apparently not repeated except in a modified form in one or two instances.

B. *Action of light on B. tuberculosis.*—Coming now directly to the effect upon the tubercle bacillus, we have scanty data to review. The earliest observations were made by Koch whose remarks at the Berlin Tuberculosis Congress, 1890, evidently made from general observations upon this subject, have found their way into so many articles and textbooks as follows:

"The tubercle bacillus is killed quite rapidly by light. A few minutes' to several hours' direct sunlight kills. Diffuse sunlight, though slower, gives the same result. Tubercle cultures set by the window die in five to seven days."

[1] *Centralbl. f. Bakt.*, 1892, 11, p. 781.
[2] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, pp. 405, 537.
[3] *Ztschr. f. Hyg.*, 1895, 19, p. 313.
[4] *Rep. Mass. State Board of Health*, 1902, p. 245.

As a result of these remarks, a general impression seems to have gone out that the tubercle is exceedingly sensitive to the influence of light, a point to which reference will be made later.

Frequent reference is also made to the work of I. Strauss,[1] who, after quoting Koch's remarks as given above, says:

"I have been equally able to assure myself that some cultures with abundant human and avian bacilli, developed upon the surface of glycerin bouillon in glass flasks, were killed after having been exposed upon a balcony for two hours to the rays of the summer sun. Besides these flasks I exposed to the sun some cultures previously dried in thin layers on some glass plates; already, at the end of half an hour, they had lost their vegetative function and their virulence."

The work of Migneco[2] also requires notice, although it is of a limited character. He smeared sputum, known to contain numerous tubercle bacilli, upon various kinds of cloth, hung the cloths so as to be exposed to sun and wind, and after various intervals of time, strips (2×5 cm.) were cut off and inoculated subcutaneously or intraperitoneally into guinea-pigs. He concludes that sunlight has a detrimental effect upon tubercle, as well as upon other bacteria, and that upon linen or woolen cloth it can withstand sunlight not more than 24 to 30 hours, provided that the layer of sputum is not too thick; also, the virulence of the bacilli is greatly weakened after 10 to 15 hours.

A number of men have carried out practical tests similar to the above, but the work scarcely requires mention here.

C. *Observations on recorded data.*—Enough has been said to show the strictly limited character of the experiments, and the totally inadequate data for any accurate conclusion to be drawn from them, so far as tubercle is concerned. It will also be observed that in all the experiments (save Migneco's), the various kinds of bacteria were exposed under glass, i. e., in glass vessels. It is a fact well known to physicists that glass reflects and absorbs a large proportion of the sun's rays, depending upon conditions. If the light strikes the surface quite obliquely, then by far the larger portion is reflected; if it strikes vertically, then a considerable amount is absorbed by the glass itself. Again, the bacteria tested have always been planted in or upon some medium (one experiment of Strauss's excepted). These media are always slightly colored or clouded, and consequently absorb even a larger proportion of the light than the glass. In some experiments, the heaping-up or clumping of the organisms, as in naturally grown cultures, or in sputum smears as used by Migneco, the data are totally invalidated save from a most limited point of view. Taken together, these various factors have discounted the effectiveness of sunlight as a germicidal agent, varying from a fourth to perhaps nine-tenths, or even more in some cases. These shortcomings in methods of work the writer has succeeded in completely overcoming, but only after a number of months of laborious work.

Hence the desirability of more adequate and accurate data concerning the action of sunlight upon the bacillus of tuberculosis goes unquestioned. It would seem, too, that a general reopening of the question of the action upon bacteria in general is not only justified, but is most highly desirable, especially in view of the rapid strides that sanitary science is making at the present time.

[1] *La tuberculose et son bacille*, 1895, p. 220.

[2] *Arch. f. Hyg.*, 1895, 25, p. 361.

A STUDY OF METHODS

Even at the beginning of the work, or rather as a result of some work done along this line in New Mexico, it was recognized that new methods were necessary, if the data are to have the highest value. This study of methods took two directions: first, an attempt to find a method that could be used with *B. tuberculosis*, since the methods usually employed fail utterly when applied to this organism; secondly, an attempt to minimize or eliminate completely the absorption factor of the interposed glass and media. These two lines of work were developed simultaneously, and it will not be convenient to separate them in the discussion, for, as will be seen, each helped to solve the other.

A. *Exposure of organism on media.*—As frequently happens, this work was begun at the most difficult point, viz., the methods for tubercle. Dorset's egg medium[1] furnished the most ready and satisfactory method of growing pure cultures of tubercle, and consequently this was selected for the work at hand. The plate method of exposure as commonly practiced was not suitable, because of the rapid desiccation incident to incubation. The most natural suggestion was to employ sloped test-tube cultures, similar to agar slopes, and expose these. Dorset's glycerine-phosphate agar was tried in the same way.

On August 19, 1905, a large number of cultures, made as suggested above, were exposed to direct sunlight for one-half, one, two, three, and five hours respectively. Two strains of tubercle bacilli were used:

Culture No. 101,[2] human type, was isolated at the local laboratory from human sputum, and was of fully average virulence.

Culture No. 110, from bovine source, was isolated by Dr. E. L. Baldwin of Saranac Lake. The virulence of this bacillus, when tested by the writer, was certainly not greater than that of No. 101, and was considerably below what is usually considered as characteristic for the bovine variety.

In this connection the descriptions of the additional varieties subsequently used may also be given.

Culture No. 102, was obtained from the Bureau of Animal Industry, U. S. Department of Agriculture, where it was used in making tuberculin. It possesses very low pathogenic powers, and grows rapidly and abundantly on egg medium.

Culture No. 113, avian, was isolated by the writer from a tuberculous hen in July, 1905. It grows more sparingly than the two human cultures, the colonies being more restricted and elevated.

[1] *Bull. Bur. Anim. Indus.*, 1904, 52, Pt. I.

[2] The numbers are those in use at the local laboratory, and have no special significance.

These bacilli, taken from young cultures, were inoculated upon the sloped media in test-tubes, and exposed to the direct rays of the sun on a wire support raised about eighteen inches from the floor. In this and all subsequent experiments the attempt was made to keep the sun's rays vertical to the surface of the medium; this was impracticable except when employed as an approximation toward the ideal. In addition to the egg and agar cultures, a watery suspension was exposed in a test-tube similarly to the others, and cultures made at intervals. Another variation consisted in rubbing the bacilli on small strips of sterile cloth, exposing these in a petri dish, and then inoculating the cloth and bacilli into an egg tube.

Substantially the same series of experiments was repeated on September 8, substituting paper for the cloth, however, and inoculating this upon egg. The results of these experiments are embodied in Table 1 below:

TABLE 1.

EFFECT OF SUNLIGHT UPON CULTURES OF *B. tuberculosis*.

EXP. NO.	DATE	NO. OF CULTURE	MEDIUM USED	RESULT OF EXPOSURE (HOURS)	
				Growth	No Growth
1	Aug. 19, '06	101	Egg	½, 1, 2, 3	5
2	" 19, '06	101	Agar	½, 1, 2,	3, 5
3	" 19, '06	101	Aqueous suspension	½	1, 2, 3, 5
4	" 19, '06	101	Cloth	½, 1, 2, 3	5
5	Sept. 8, '06	101	Egg	½, 1, 1½, 2, 3	.....
6	" 8, '06	101	Aqueous suspension	½	1, 1½, 2, 2½, 3
7	" 18, '06	101	Paper	½	1, 1½, 2, 2½, 3
8	Aug. 19, '06	110	Egg	½, 1, 2, 3, 5	.....
9	Sept. 8, '06	110	"	½, 1, 1½, 2, 3	.....
10	" 8, '06	110	Paper + Moisture	½	1, 1½, 2, 3

The data in this table showed a number of interesting points:

a) The method of exposing test-tube cultures is plainly unsuited to this purpose. This is readily understood when it is recalled that many of the bacilli are worked into the egg during the process of inoculation, and are consequently materially protected. The cylindrical surface of the tube tends to concentrate the light in a line and thus produce unequal intensities upon different portions of the surface of the medium. Consequently, this method was immediately abandoned.

b) The method of exposing watery suspensions appeared quite promising, but it was open to the objection of unequal distribution of light, which is not wholly remedied by placing the suspensions in potato or petri dishes. In addition, there was a marked tendency for the culture to sediment.

c) The method of spreading the culture on cloth and subsequently inoculating upon egg was also unsuitable, for the reason that the culture worked into the interstices of the cloth and so received protection. Data thus obtained are quite worthless, since we have no means of knowing how much of the effect to ascribe to sunlight and how much to the factor of protection.

d) The method of spreading the pure culture upon sterile paper slips and then exposing them in petri dishes gave the same results as the watery suspensions, and at the same time eliminated the objectionable features of that method, i. e., the unequal distribution of light. Accordingly, this method was temporarily adopted in the work, and considerable data collected, before any advance was made.

B. *Exposure of organism on strips of paper under glass.*—The strips of paper used were about 1 × 5 cm. area; they consisted of ordinary, sized paper and were sterilized by heat before using. Such strips of paper are difficult to inoculate when placed in a glass vessel; but this difficulty can be overcome by placing a piece of cloth in the bottom of the dish and laying the paper upon it; the cloth holds the paper in place both during the inoculation and the exposure. Watery suspensions spread very readily upon such sized paper, serving better than many materials subsequently tried.

In applying this method, several important questions arose, which require discussion:

a) *How does the quantity of culture inoculated upon the paper influence the result?* An early instance of the possible variation in result due to this factor was observed. Among a series of cultures, exposed September 8, were two sets on paper slips: one set showed that tubercle was killed between one and one-half and two hours; the other set between one-half and one hour. At that time the method of inoculating the paper slips was to rub upon them some of the culture by means of a stiff platinum wire. Plainly, the amount of culture transferred to the paper varied within wide limits, even when special care was exercised. Portions from different parts of the culture tube would spread very differently, and necessarily required

different periods of exposure to kill, as shown by the instance cited above. Other instances of such variation were observed, but the details need not be given, for the point is perfectly plain. Suffice it to say that steps were immediately taken to remedy this defect. These steps were as follows:

(1) Instead of taking the culture direct, a suspension in plain water was first made, then a small loopful of this suspension was spread upon the sterile paper slips. With most species of bacteria, this method works quite satisfactorily, but this is not always the case with tubercle. Cultures of tubercle vary so widely in their physical condition, depending upon their age and the medium employed, that all grades ranging from a soft, friable to a hard, resistant mass are obtained. These do not work up equally well into a homogeneous suspension, but are apt to produce flocculi which may readily be carried over to the paper. It is obvious that the germs within or under such a lump are protected to a considerable degree from the influence of sunlight. The effort to overcome this difficulty led to the second step:

(2) The filtration of the suspension through glass wool. Necessarily this must be done under aseptic conditions to prevent contamination. The suspension so obtained is much more uniform in character, all the gross lumps and fatty flocculi being removed. It does not completely remove the microscopic lumps consisting of 10 to 50 bacilli, and to break these up still further it is advisable to employ a shaking machine for some time. However, quite uniform results can be obtained without the additional shaking.

b) How is the inoculation of the media with the cultures on paper to be accomplished? This was done in a simple and surprisingly satisfactory manner by placing the paper by means of a sterile forceps on the egg slope. At first an attempt was made to dislodge the bacilli from the paper, but it was soon found that the culture grew admirably upon the paper if it was carefully pressed upon the medium and sufficient fluid was present to moisten the paper. The nutrient medium filters through the paper and nourishes the bacteria, which grow in a wholly satisfactory manner, as shown in Pl. 2. The method is applicable to agar slopes also, if care is taken to have some expressed water present; but obviously, old culture media work unsatisfactorily. When experimenting with bacteria which grow well in broth or other liquid media, the method becomes the acme of simplicity; the slip of paper is dropped into the tube of bouillon or milk, placed in the incubator, and the characteristic growth phenomena noted.

c) Is it possible to diminish the absorption of light by substituting a more suitable medium for the glass? Pure quartz plates would absorb less light than common glass, but such plates are expensive and practically out of the question. Gelatin films and mica plates next suggested themselves, and these were accordingly tried. On October 28, cultures of Tubercle No. 101 were exposed on paper under these covers, and also under glass. The results showed that:

1. Under glass, tubercle was killed between 0 and ½ hrs.
2. " mica, " " " " 1½ " 2 "
3. " celluloid, " " " " ½ " 1 "

Apparently there was no advantage to be gained by the use of either mica or celluloid. Before the test was repeated, the problem was solved by rendering it non-existent as will be shown later. The whole question might well have been ignored, but for the fact that it illustrates nicely one of the steps in the progress of the work.

d) What part of the bactericidal effect is due to desiccation? Considerable indirect evidence, gained from the earlier experiments, indicated that it was a minor, if not a negligible, factor.

A direct experiment, made December 30, gave the following results:

TABLE 2.

	Organism	Moisture Added	Result of Exposure (Min.)	
			Growth	No Growth
1.....	*B. coli*	−	..	3, 6, 10, 15, 20, 30
	"	+	3	6, 10, 15, 20, 30
2.....	*B. typhosus*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
3.....	*M. tetragenus*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30
4.....	*B. diphtheriae*	−	..	3, 6, 10, 15, 20, 30
	"	+	..	3, 6, 10, 15, 20, 30

It should be noted that the above exposures were made on an unusually clear day, which accounts partially for the short time required to kill the cultures. While, in one instance, the moisture seems to have prolonged the life of the organism, yet, taken as a whole,

the total time involved was so short as to make it quite doubtful whether this was a determining factor.

In another experiment, April 7, an attempt was made to exaggerate conditions by surrounding one set with conc. sulphuric acid, thus rendering the air quite dry, while the other set had sufficient water added to cause a film of moisture to gather under the cover. Under these conditions, both sets were killed within five minutes, the shortest time of exposure.

It appears highly probable, therefore, that the presence of moisture is neither instrumental in killing, nor in greatly prolonging the life of the cultures. Indeed, as we shall see, the method finally adopted totally removes this question from the main problem.

e) Does the character of the paper used influence the effect of sunlight in killing the culture? It was stated at the beginning that the paper used was a common, sized writing-paper. It is conceivable that in the process of manufacturing, especially in bleaching, some antiseptic properties were communicated to the paper. To test this possibility, *B. coli* was exposed to sunlight on eight different substances (March 15) with the following results:

TABLE 3.

Trial	Material Employed	Result of Exposure (Min.)	
		Growth	No Growth
1	Ordinary, sized paper	2, 7	12, 20
2	Filter paper	2, 7, 12, 20	.....
3	Black porous paper	2, 7, 12, 20	.....
4	Parchment paper	2, 7	12, 20
5	Aluminum foil	2, 7, 12, 20	.....
6	Mica	2, 7, 12	20
7	Glass	2, 7, 12	20
8	Wood	2, 7, 12, 20	.....

These results appear somewhat surprising at first; but, upon analysis, the reasons for the differences observed are quite plain. The filter paper, the porous paper, and the wood (a soft pine) are sufficiently porous to permit the individual bacilli to enter with the water and thus become sheltered; the aluminum foil, on the other hand, did not permit the making of a uniform spread, the culture drying in patches, which caused a heaping-up effect with consequent protection.

Similar experiments with the same organism gave the following:

TABLE 4.

Trial	Date	Material Employed	Results of Exposure (Min.)	
			Growth	No Growth
1	March 16	Paper previously used	2	5, 10, 15, 20
2	" 16	Parchment	2, 5, 10, 15, 20	.....
3	" 16	Mica	2, 5, 10	15, 20
4	" 16	Glass	2, 5	10, 15, 20
5	" 17	Paper previously used	3, 6	10, 15, 20
6	" 17	Parchment	3, 6, 10, 15	20
7	" 17	Mica	3, 6, 20	10, 15
8	" 17	Glass	3, 6, 20	10, 15
9	" 17	Filter paper	3, 6, 10, 15, 20, 30	.....

The filter-paper test requires no further comment. Glass shows the same phenomenon as aluminum when not absolutely free from grease; this tendency is shown by the erratic results that it gives. The parchment invariably became roughened and warped in the sterilizing process, which renders it unsuitable for the purpose in hand. There is left to consider only the mica. This was a genuine disappointment to the writer, for the culture spread very evenly upon it. Apparently this should be an ideal substance to use and would serve as a fair check upon the paper method. Upon still other repetitions this continued to give erratic results, and the explanation undoubtedly is to be found in the fact that the surface of a small piece of mica is very imperfect, many cracks extending inward from the cut edges; in these cracks the bacteria evidently found shelter.

Thus the tests fell short of their object, and merely proved the inferiority of these substances for this purpose. Sized paper gives a beautiful spread, as compared with these; there is little tendency to creep into pores, for these are filled by the sizing, and the results are as uniform as could well be expected.

The question with which we started is answered in a measure, at least, by the fact that innumerable control experiments always gave growth, even when the culture remained exposed for several days. Again, *B. tuberculosis* grew admirably upon these papers (see Pl. 2), which certainly contra-indicates the presence of antiseptic principles in material quantities. Finally, as will be seen, cultures exposed without the presence of paper are killed in even shorter periods of time.

C. Direct exposure of organisms.—Thus far, the object has been to minimize the absorption factor, and in this way approach more

closely to the true disinfecting action of sunlight. A very material advantage has been gained, *the exclusion of the nutrient medium and the accompanying absorption.* Incidentally this affords another advantage also, viz., the elimination of possible by-products which may be formed in the medium, such as hydrogen peroxide, ozone, organic peroxides, etc., which might aid in killing the cultures and thus complicating conditions, and rendering the action of the sunlight still more problematical. Indeed, it was not certain that any of the disinfecting action could positively be ascribed to the direct action of the sun's rays. To have eliminated these side reactions and at the same time have a method that is applicable in general, for *B. tuberculosis* included, seemed for a time to present the limit of perfection attainable. Nevertheless, the problem of the elimination of all absorption to obtain the true effect of sunlight was not forgotten.

This problem was attacked at the most difficult point. It was known that the tubercle bacillus could be exposed directly, and by use of a suspension which could be inoculated into animals the contaminating factor would be practically negligible. Accordingly, this experiment was tried. A suspension (heavier than usual), of tubercle No. 101, was allowed to evaporate in the bottom of a petri dish, and exposed, uncovered, to the direct rays of the sun. Four or five drops of the suspension were used to insure sufficient material for the inoculation. At the same time, two of the watery suspensions were also exposed in petri dishes, one being covered and the other not, the latter to serve for comparison. These cultures were exposed for one-half, one, one and one-half, and two hours, when they, together with controls kept in the dark, were injected respectively into guinea-pigs, the inoculations being intraperitoneally in all cases.

This experiment was unfortunate in two respects; firstly, the day chosen gradually developed into a hazy one, so that the sunlight was much diminished in power; secondly, a number of the test animals died from an epidemic of disease that affected the stock of guinea-pigs on hand. The guinea-pigs inoculated with the cultures exposed for the longest time, i. e., two hours, all developed tuberculosis and died. The living tubercle bacillus was recovered in pure culture from several animals of this series. The fact that the cultures were alive after two hours' exposure is explained by the diminished sunlight, on

one hand, and by the thickness of the film and the tendency of the suspensions to sedimentate, on the other.

Several months later it occurred to the writer that organisms could be exposed without cover, and all absorption of light be entirely avoided. This method gave most gratifying results as follows:

A suspension of *B. coli* was made, and a loopful spread in a circle in the bottom of sterile petri dishes. (See Pl. 3.) While exposed to the sun, the dishes were uncovered; when taken in, they were again covered, and a layer of lactose litmus agar, which was melted and cooled to 45° C., was spread over the exposed film. The cultures were placed in the incubator for 24 and 48 hours. The first two sets of plates showed that *B. coli* was killed within two minutes. On March 23, the same method showed *B. coli* to be killed in between two and four minutes. March 31, *B. diphtheriae* was killed in between two and five minutes.

The advantages in selecting *B. coli* in the above and subsequent experiments are these: (*a*) It is not apt to be contaminated with fresh colon bacilli from the air; (*b*) it grows well as 37°–39° C., while the common air forms that might contaminate the plate do not, as a rule, develop at this temperature; (*c*) it grows well on lactose litmus agar, while most bacteria do not; (*d*) it gives red or acid colonies, on this medium, while this would rarely be the case with air germs; (*e*) if any question arose as to whether a given colony was *B. coli* or not, cultures in glucose bouillon fermentation tubes and in milk would decide the matter, in nearly all cases, the proper gas formula being especially helpful in reaching a decision.

That this method, with suitable variations in media, etc., can be applied to all bacteria, goes almost without saying. Glucose agar will be found best adapted to all acid-producing species, in which case the circle or other character used for the film will be most helpful (see Pl. 3); if only occasional colonies appear they should be isolated and subjected to confirmatory tests. Species that require a highly proteid medium, like *B. tuberculosis*, may be planted upon strips of sterile paper, and after exposure inoculated upon egg or blood serum. Necessarily, a sufficient number of cultures must be made so that one or more of each lot for a given period will come through uncontaminated; on a quiet day this is not at all difficult to accomplish.

D. *Comparison of methods.*—In a certain way, the methods employed have been compared with each other; but it will now be advantageous to make a more direct comparison, especially of data secured with this object in mind. For this purpose only three methods have been considered available; viz.: (*a*) the agar-plate method; (*b*) the method of paper cultures under glass; and (*c*) the method of direct exposure of the bacterial films, no glass or other

medium being interposed. The method of exposing suspensions has not been considered because of its numerous disadvantages.

On March 23, an experiment with *B. coli* partly covering these points gave the following:

1. On paper, under glass, *B. coli* was killed in between 11 and 15 min.
2. " glass, " " " " " " 10 " — "
3. " paper, no cover, " " " " " " 1 " 2 "
4. " glass, " " " " " " 2 " 4 "

The absorption of light by the glass is considerable, and in this instance prolonged the life of the bacterium considerably beyond that required to kill without any interfering medium.

April 11, another series with *B. coli* gave the following:

1. On glass, no cover, killed within 2 min.
2. In agar plate, glass cover, 25 per cent were killed in 10 min.
   85 " " " " " 20 "
   95 " " " " " 30 "
   100 " " " " " 45 "

These plates are shown in composite in Pl. 3, Fig 2.

A repetition of the above experiment with other bacteria gave the following results:

TABLE 5.

Organism	Date	Time Required to Kill	
		By Direct Exposure	In Agar Plates
B. cholerae suus	Oct. 12, 1906	Between 5 and 10 min.	In 40 min. 10 per cent killed
B. phosphorescens	" 25, 1906	" 1 and 2 "	" " " 95 " "
B. prodigiosus	" 30, 1906	" 5 and 10 "	" " " 70 " "
B. Friedländer	Nov. 7, 1906	" 0 and 1 "	" 20 " 75 " "
B. typhosus	" 9, 1906	" 5 and 10 "	" 40 " 100 " "
			" 45 " 90 " "

The time required to kill bacteria is reduced to at least $\frac{1}{15}$th, and possibly to $\frac{1}{20}$th, of the time required to kill by the old or plate-culture method. These data could be largely extended, but they are sufficient for the present purpose, viz., to show the great advantage possessed by the method of direct exposure. They indicate also the unreliable character of the data thus gathered by the earlier observers, and which have, at most, only a relative value, for only a $\frac{1}{10}$th or $\frac{1}{20}$th of the sun's disinfecting action has been employed in securing them.

E. *Observations on methods.*—Before leaving the matter of methods a number of minor points may appropriately be mentioned here.

a) Apparently most investigators have paid little heed to the meteorological conditions, or have failed to mention the details. In the present investigation, only cloudless sunlight has been employed, unless otherwise stated. The time has been as near midday as practicable, most of the determinations having been made between 11 and 1 o'clock. In this work the accompanying table has been of material service.

TABLE 6.

COMPARATIVE LIGHT VALUES* (EXPRESSED IN SECONDS).

Time of Day	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
8:00	10.0	6.0	3.0	2.0	1.7	1.7	1.5	1.7	2.0	3.0	4.0	9.0
9:00	4.0	4.0	2.0	1.5	1.2	1.2	1.2	1.5	1.5	1.7	3.0	3.0
10:00	2.5	2.0	1.5	1.7	1.0	1.0	1.0	1.0	1.0	1.5	2.0	2.0
11:00	2.0	1.7	1.2	1.0	0.7	0.6	0.5	0.7	1.0	1.2	1.7	1.5
12:00	1.7	1.5	1.0	1.0	0.5	0.3	0.2	0.5	0.5	1.0	1.5	1.7
1:00	1.7	2.0	1.0	1.0	0.7	0.5	0.5	0.5	1.0	1.2	1.5	2.0
2:00	2.5	2.7	1.7	1.2	1.0	0.7	1.0	1.0	1.2	1.5	2.0	2.5
3:00	4.5	3.5	2.0	1.5	1.2	1.2	1.2	1.2	1.5	2.0	2.5	3.5
4:00	9.0	5.0	4.0	2.0	1.5	1.5	1.5	1.7	2.0	2.7	5.0	8.0
5:00		60.0	20.0	4.0	2.7	2.2	2.2	2.5	3.0	6.0	20.0	80.0
6:00				20.0	15.0	5.0	4.0	5.0	10.0	40.0	70.0	
7:00					80.0	20.0	15.0	60.0				

* By Lieutenant S. W. Very, U. S. N. Printed in *Am. Annual of Photography and Photographic Times Almanac* for 1899.

Table 6 gives the relative light values for photographic purposes expressed as time necessary to expose the plate. All the work has been done at times falling between 0.2 and 1 in this table, thus giving the most intense light obtainable. This affords a certain standard for the work; absolute values, if attainable, would scarcely serve a better purpose.

b) During the warmer days the temperature was taken to guard against the possibility of killing the cultures by heat. It is evident that, in the short exposures as finally practiced, temperature is not a determining factor in any case.

c) Young cultures were used for the exposures; these were usually one or two days old, and rarely more than a week old. In the case of tubercle, the cultures varied from one to two months in age. Controls always served as an additional check upon the vitality of the culture.

EXPERIMENTAL RESULTS.

The striking difference in the results obtained by the old methods as compared with those here suggested is best illustrated by a direct comparison of results for specific bacteria.

B. coli.

Dieudonné[1] found that agar plate cultures of *B. coli* when exposed to midday sun were killed in 1 to 1½ hours. Buchner[2] found that aqueous suspensions were sterile after 1 to 1½ hours' exposure.

In comparison with these, the writer found the following:

TABLE 7.

Date	Method of Exposure	Result of Exposure (Min.)	
		Growth	No Growth
March 20, 1906..	On glass, uncovered	.....	2, 4, 6, 8, 10, 15
" 23, 1906..	" " "	½, 1, 2	4, 7, 10
" 23, 1906..	" paper, "	½, 1	2, 4, 7, 10
April 7, 1906....	" glass, "	2	5, 10, 20, 40
" 11, 1906....	" " "	.....	2, 5, 10, 20, 30

The difference between the above results and those obtained by former investigators is most remarkable.

B. typhosus.

Kedzior[3] found that if a 5 c.c. bouillon culture of typhoid was exposed to sunlight, it was not killed in 4¼ hours; even a 1 c.c. culture was not killed in 5⅜ hours.

By direct exposure this organism was killed as follows:

TABLE 8.

Date	Method of Exposure	Results of Exposure (Min.)	
		Growth	No Growth
April 27.....	On glass, uncovered	2, 5	10, 15, 20
May 10.....	" " "	.....	2, 5, 10, 20, 30
" 15.....	" " "	2	5, 10, 20

The results of April 27, are nearer the others than the figures would seem to indicate, for only a couple of colonies remained on the plate exposed for five minutes. When compared with Kedzior's results, it is seen that the time necessary to kill typhoid by direct exposure is reduced to $\frac{1}{60}$ or less. The advantages of the method of direct exposure are evident.

B. dysenteriae (Shiga).

Quite similar to *B. coli* and *B. typhosus* are the results for *B. dysenteriae:*

TABLE 9.

Date	Method of Exposure	Results of Exposure (Min.)	
		Growth	No Growth
April 22.....	On glass, uncovered	.....	2, 5, 10, 20, 30
May 9.....	" " "	.....	2, 5, 10, 15, 20
" 10.....	" " "	2	5, 10, 20, 30

[1] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, p. 405.

[2] *Centralbl. f. Bakt.*, 1892, 11, p. 781.

[3] *Arch. f. Hyg.*, 1899, 36, p. 323.

S. cholerae-asiaticae.

In an experiment similar to that with typhoid bacilli, Kedzior found that a 5 c.c. bouillon culture gave a good growth after 4 hours, and a 1 c.c. culture was killed in 5⅜ hours.

Direc exposure gives the following:

TABLE 10.

Date	Method of Exposure	Results of Exposure (Min.)	
		Growth	No Growth
April 11.....	On glass, uncovered	.....	2, 5, 10, 20, 30
" 27.....	" " "	.....	2, 5, 10, 15, 20
May 19.....	" " glass cover	1, 2	6, 10, 15

B. diphtheriae.

Kedzior also exposed cultures of diphtheria and found them to be killed in 1½ to 2½ hours.

By direct exposure, this organism was killed as follows:

TABLE 11.

Date	Results of Exposure (Min.)	
	Growth	No Growth
March 31.....	½, 1, 1½, 2	5
April 27	2	5, 15, 20
May 10.....	2	5, 10, 20, 30

The Pus Cocci.

No one seems to have experimented with these forms, hence, no comparison is possible. The results obtained by direct exposure are as follows:

TABLE 12.

Date	Organism	Results of Exposure (Min.)	
		Growth	No Growth
April 2.....	*M. pyog. aureus*	3, 6, 10	15, 25
" 22.....	" "	2, 5	10, 20, 30
May 15.....	" "	2, 5	10, 20
April 7.....	*M. tetragenus*	2	5, 10, 20, 40
" 11.....	"	2	5, 10, 20, 30
" 22.....	"	2	5, 10, 20, 30
May 10.....	"	.....	2, 5, 10, 20, 30

In the earlier work two tests were also made with *M. pyog. albus*, but at that time the short period of exposure necessary to kill was not appreciated, and so the results are not very helpful. In the first trial, it was killed within 30 minutes, and in the second within 10 minutes.

B. prodigiosus.

Dieudonné[1] studied this organism because he thought it would be especially sensitive to light. He found it to be killed in between 1½ and 2½ hours in gelatin and agar-plate cultures.

Direct exposure gave the following:

TABLE 13.

DATE	RESULTS OF EXPOSURE (MIN.)	
	Growth	No Growth
April 27.....	.....	2, 5, 10, 15, 20
May 11.....	1, 2	5, 10, 15

It may be worth noting that, on the two-minute plate of May 11, the color production was not diminished, although the number of colonies was greatly reduced.

B. pyocyaneus.

Kedzior worked with this organism and found it was killed in agar plates in between 2½ and 3½ hours. By the method of direct exposure, the writer found it was destroyed as follows:

TABLE 14.

DATE	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
		Growth	No Growth
April 7.....	On glass, uncovered	.....	2, 5, 10, 20
" 11.....	" " "	2	5, 10, 20, 30
May 9.....	" paper, "	.....	2, 5, 10, 15, 20
June 30.....	" glass, "	½, ½, 1, 2	4, 10

Two other chromogens were tried, with the following results:

TABLE 15.

DATE	ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE (MIN.)	
			Growth	No Growth
April 2.....	*Sar. aurantica*	On glass, direct	3, 6, 10, 15, 25	.....
May 15.....	" "	" "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60	90, 120
" 15.....	A pink air-micrococcus	" "	2, 5, 10, 20	.....
" 19.....	" "	" " under glass	10, 20, 30, 40, 60	.....
" 28.....	" "	" " direct	60, 90	120

These two organisms show a higher order of resistance to the influence of sunlight than those heretofore considered. (See Pl. 3, B.) Indeed, they constitute a group by themselves, to which, presumably, many of the bacteria found in the air belong. Whether the resistance they exhibit is due to a failure to break up the groups, or to a sporelike condition (so-called arthrospore) of the bacterium, remains uncertain. But, from the nature of the suspensions used, and from the character of the growth in the plates, the writer is inclined to hold the latter view.

[1] *Arb. a. d. kais. Gsndhtsamt.*, 1894, 9, p. 405.

It is fortunate that the pathogenic organisms show no such powers of resistance, or the problem of coping with them would be materially more difficult.

B. tuberculosis.

Coming now to the bacillus of tuberculosis, which is the chief factor in the present investigation, one finds practically no previous tests that are satisfactory, with which to compare the results. Koch's remarks are seemingly based upon casual observation, which, however, indicated that the organism was highly sensitive to light, presumably more so than other bacteria. Strauss came close to the truth in his trial by exposing a film on glass plates, which he found was killed in 30 minutes. Other workers have used sputum with variable results, ranging from 24 to 30 hours, up to days or weeks.

The earlier results when the bacillus was exposed on egg medium have been given; they are relatively high, varying between two and five hours in time necessary to kill; in aqueous suspensions it was killed in ½ to 1 hour. The later results, when no culture medium was used in the exposure, are as follows:

TABLE 16.

DATE	NO. OF ORGANISM	METHOD OF EXPOSURE	RESULTS OF EXPOSURE	
			Growth	No Growth
September 8, 1905....	101	On paper, under glass	½, 1, 1½	2, 3, 4, 5, 6 (hrs.)
" 8, 1905....	110	+moisture, on paper, under glass	½	1, 1½, 2, 2½, 3 (hrs.)
" 8, 1905....	101	" " " " "	½	1, 1½, 2, 2½, 3 (hrs.)
October 28, 1905....	101	On paper, under glass	.....	½, 1, 1½, 2, 2½, 3 (hrs.)
November 11, 1905....	101	" " " "	½, 1, 1½	2, 2½, 3, 4 (hrs.)
" 30, 1905....	101	+moisture, on paper, under glass	10, 20	30, 45, 60 (min.)
December 24, 1905....	101	On paper, under glass	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	101	" " " "	½	1, 2, 3 (hrs.)
" 26, 1905....	101	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	102	" " " "	10	20, 30, 45, 60 (min.)
" 26, 1905....	110	" " " "	.....	10, 20, 30, 45, 60 (min.)
" 26, 1905....	113	" " " "	10	20, 30, 45, 60 (min.)
April 4, 1906....	101	" " direct	5, 10, 15	20 (min.)
" 11, 1906....	102	" " "	.....	5, 10, 15, 20 (min.)

In the work prior to December, the data showing greatest longevity were obtained, as previously noted, by rubbing the pure culture upon the paper and then making the exposure; the December data were secured from aqueous suspensions inoculated upon paper, while those in April and later were films from suspensions, dried on paper and exposed to the sun directly, i. e., without glass or other intervening medium.

Leaving out of account the earlier results, as not being satisfactory, it appears that the method of direct exposure gives as consistent and similar results with tubercle as with other non-spore-bearing bacteria; it is more sensitive than some, but less so than *B. coli*, for example. Too close comparisons are not admissible here, for the time is so short that slight but unavoidable variations may place the result on one side or the other. Apparently tubercle is not especially sensitive to light. This may be only apparently so, for, as was mentioned earlier, homogeneous suspensions are not so readily obtained with tubercle as with other bacteria, and, as a result, clumping, with consequent protection, takes place.

In this connection, another experiment will be of interest. A sample of sputum, containing numerous tubercle bacilli and also cocci, etc., was spread in thin films on papers and exposed under glass in the usual way. In the cultures where development took place, *M. pyogenes aureus* and *albus* were present; but in those that remained free from contamination, tubercle failed to develop. The periods of exposure were 10, 20, 30, 45, and 90 minutes. A repetition of this experiment gave the same results. This would seem to indicate that the tubercle bacillus is not more resistant to the action of light than were the other bacteria that chanced to be present in the sputa.

The details of an experiment made on April 4 will be interesting and instructive, especially in helping to explain the variable results obtained with tubercle, and also its apparent endurance. A watery suspension of a culture of tubercle No. 101 was employed. This suspension was filtered through a layer of glass wool, and subsequently inoculated upon paper strips. These strips were sealed at one end to the bottom of a petri dish by means of hot paraffin. After inoculation, they were exposed, uncovered, to direct bright sunlight between 12:28 and 12:54 P. M. The results are given in Table 17.

It is seen that none of the five-minute exposures was killed; that two out of four of the 10-minute exposures grew, one showing only a single colony; also two from the 15-minute lot showed each a single colony; and that none of the 20-minute cultures grew. Two controls gave excellent growth. Although the suspension from which these inoculations were made was filtered, and a macroscopic examination showed decided improvement in homogeneity as compared with the original, yet a microscopic examination still revealed occasional clumps of bacilli aggregating 10–50 in number. Undoubtedly it is due to this tendency to clump that tubercle gives such varying results, and a false impression is obtained in regard to its true resistance to sunlight, which is probably not greater than that of the common pus cocci.

TABLE 17.

RESULTS WITH TUBERCLE, BY DIRECT EXPOSURE

Trail No.	Time of Exposure	Result
1	5 min.	Good growth
2	5 "	Four colonies
3	5 "	Fair growth
4	5 "	Slight growth
5	10 "	No growth
6	10 "	One small colony
7	10 "	No growth
8	10 "	Good growth
9	15 "	Contaminated
10	15 "	One colony
11	15 "	"
12	15 "	No growth
13	20 "	" "
14	20 "	" "
15	20 "	" "
16	20 "	" "
17	Control	Good growth
18	"	" "

An experiment was made with Möller's grass bacillus, which is acid-fast and simulates tubercle very closely, except that it grows at room temperature on common media. In this case it was not killed in 15 minutes, on May 11, at midday. Here clumping was marked, and was accompanied by a corresponding longer period of resistance to sunlight.

It was stated by Koch that cultures of tubercle are killed in five to seven days in diffuse light. This point was also tested, cultures on paper slips being exposed for comparison. The exposures were made directly in front of a north window in the laboratory next to the window-sill, all the cultures being under glass.

In this series the pure culture was rubbed onto the paper slips. The sunlight afforded by a number of days was necessary for these long exposures. While the results are too few for drawing any positive conclusions, they do not indicate any extraordinary sensitiveness to diffuse light on the part of tubercle, for the culture of October 21 required six days of exposure and then was not killed. The cultures

TABLE 18.

Date	Culture	Method of Exposure	Results of Exposure (Hours)	
			Growth	No Growth
September 7, 1905.....	No. 101	On egg medium	2, 5, 9½, 15, 22½, 30	.....
" 7, 1905.....	" 101	On paper+moisture	2, 5, 9½, 15, 22½, 30	.....
October 21, 1905.....	" 101	On egg medium	25, 30, 35, 40, 45, 50	.....
" 21, 1905.....	" 101	On paper	.....	25, 30, 35, 40, 45, 50
" 21, 1905.....	" 101	On paper+moisture	25	30, 35, 40, 45, 50
" 21, 1905.....	" 110	On paper	25	30, 35, 40, 45, 50

on paper slips show that diffuse light does have an effect; this is not due to desiccation, for when moisture was added the result was similar.

Taking the results for tubercle as a whole, there is no good reason to ascribe to this organism any special powers in the way of resistance or lack of resistance to sunlight; in fact, it possesses about the same order of reistance as the other nonsporogenous pathogenic bacteria.

A number of experiments were carried out to determine which rays acted bactericidally upon *B. tuberculosis*. The method adopted was to employ the paper slip cultures under a single pane of red, green, or blue glass. These glass panes were found, however, not to give pure monochromatic light, for the red filtered through the blue, and the blue through the red, while both filtered through the green. Under these circumstances, Tubercle No. 101 gave the following results:

TABLE 19.

Trial	Date	Method of Exposure	Result of Exposure (Min.)	
			Growth	No Growth
(a).....	May 3	Under colorless glass	.....	5, 10, 15, 20, 30, 45
(b).....	" 3	" red "	5, 10, 15, 20	30, 45
(c).....	" 3	" green "	5, 10, 15, 20, 30	45
(d).....	" 3	" blue "	5	10, 15, 20, 30, 45
(e).....	" 17	" colorless "	5	10, 20, 30
(f).....	" 17	" red "	5, 10	30
(g).....	" 17	" green "	5, 10, 20, 30	.....
(h).....	" 17	" blue "	5, 10	20, 30

These experiments indicate that it is the violet end of the spectrum that is fatal for tubercle, just as for other forms.

Since the data which have been recorded in the foregoing tables are distributed throughout the paper, it appears wise to collect them in a single table for convenience of reference and comparison. The table is as follows:

TABLE 20.

SUMMARY OF RESULTS OF EXPOSING BACTERIA TO DIRECT SUNLIGHT

Date	Organism	Method of Exposure	Limits of Life (Min.)
March 20.....	*B. coli*	Direct	0 and 2
" 23.....	"	"	2 " 4
" 23.....	"	"	1 " 2
April 7.....	"	"	2 " 5
" 11.....	"	"	0 " 2
May 11.....	" (No. 547)	"	2 " 5
" 11.....	" (No. 711)	"	2 " 5
" 11.....	"	"	2 " 5
April 27.....	*B. typhosus*	"	5 " 10
May 10.....	"	"	0 " 2
" 15.....	"	"	2 " 5
April 22.....	*B. dysenteriae*	"	0 " 2
May 9.....	"	"	0 " 2
" 10.....	"	"	2 " 5
April 11.....	*S. cholerae-asiaticae*	"	0 " 2
" 27.....	"	"	0 " 2
May 19.....	"	"	2 " 6
March 31.....	*B. diphtheriae*	"	5 " 10
April 27.....	"	"	0 " 2
May 10.....	"	"	2 " 5
April 2.....	*M. pyog. aureus*	"	10 " 15
" 22.....	" "	"	5 " 10
May 15.....	" "	"	5 " 10
April 7.....	*M. tetragenus*	"	2 " 5
" 11.....	"	"	2 " 5
" 22.....	"	"	2 " 5
May 10.....	"	"	0 " 2
April 27.....	*B. prodigiosus*	"	0 " 2
May 11.....	"	"	2 " 5
April 7.....	*B. pyocyaneus*	"	0 " 2
" 11.....	"	"	2 " 5
May 9.....	"	"	0 " 2
June 30.....	"	"	2 " 4
April 2.....	*Sar. aurantica*	"	25 " ..
May 15.....	"	"	20 " ..
" 19.....	"	Under glass	60 " ..
" 28.....	"	Direct	60 " 90
" 15.....	Pink air-coccus	"	20 " ..
" 19.....	" "	Under glass	60 " ..
" 28.....	" "	Direct	90 " 120
" 12.....	Möller's grass bacillus	"	15 " ..
December 24...	Tubercle 101 (human)	Under glass	0 " 10
" 26...	"	" "	30 " 60
" 26...	"	" "	0 " 10
April 4.....	" 101	Direct	15 " 20
December 26...	" 102 (human)	Under glass	10 " 20
April 11.....	" 102 (human)	Direct	0 " 5
December 26...	" 110 (bovine)	Under glass	0 " 10
" 26...	" 113 (avian)	" "	10 " 20
April 23.....	" 113	Direct	15 " ..

PRACTICAL BEARINGS OF THE RESULTS.

In reading the publications of the workers in this field, especially those of the earlier investigators, one notices that they expected rather more disinfectant action from the sunlight than was found; and so there seems to be something of a tone of disappointment. However this may be, it is certain that the results they obtained fell far short of doing justice to the disinfecting action of the sun's rays. While this action has been considered important from a sanitary view-

point, it is certain that it is much more powerful than previous results have indicated. Were it not for this powerful repressive agent, coupled especially with the repressive action of desiccation, it seems, indeed, that our chances for waging a successful war against disease and other objectionable bacteria would be slight; but when we realize that 2 to 10 minutes of active sunlight are sufficient to kill them when directly exposed, we can readily understand how the vast majority of all such bacteria are effectively destroyed, and only an infinitesimal number remains. This gives sanitary science new hopes and fresh courage with prospects of the highest success.

These results explain, in a measure, the advantages of a dry climate, such as the western and southwestern portions of the United States possess, where, due to the dryness and the superabundant sunshine, most bacteria, and especially the non-spore-bearing disease germs are quickly destroyed. Above all, they emphasize the importance of well-lighted and ventilated houses. The sunlight is a friend and protector of our welfare and should not be barred from our homes by shutters and heavy shades; for there is truth in the Italian saying: "Where sunlight enters not, there the physician goes."

CONCLUSIONS.

The conclusions arrived at may then be summarized as follows: The methods heretofore employed in testing the bactericidal action of sunlight do not seem to be well suited for this determination, since the results do not indicate the full power of this agent.

The light is absorbed by the medium in which the bacteria are planted, and the glass cover both absorbs and reflects a considerable portion of the effective rays. A more suitable method consists in planting the bacteria upon glass or paper and exposing directly, i. e., without glass or other cover, to the sun's rays. By this method most of the non-spore-bearing bacteria, including *B. tuberculosis*, *B. diphtheriae*, *B. typhosus*, *S. cholerae-asiaticae*, *B. coli*, *B. prodigiosus*, and others, are killed in a remarkably short period of time, varying from 2 to 10 minutes. This time is considerably lengthened if the suspension used is not homogeneous and the bacteria consequently become clumped or bunched in the film. Certain saprophytic bac-

PLATE 2.