The Influence of Ionised Air on Bacteria.

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[Plates 7—12.]

1. On exposing surface films of bacteria upon agar to the electric wind from charged needle points, with subsequent incubation, it is found that the wind from negatively charged points has more bactericidal effect than that from points positively charged.

In a recent paper* it was suggested that there should be this difference in consequence of the positive charge found to be associated with fresh vegetable cells, and further, that negatively charged air might prove useful in the treatment of tuberculous disease of the lung by retarding the growth of the bacteria. Before attempting direct trial of the latter point it was necessary to show that negative electric charge does inhibit bacterial growth in general under laboratory conditions of culture, and the present work was undertaken for this purpose.

2. The most suitable method of ionising air on a large scale either positively or negatively is by unidirectional point discharge at high potential. The voltage gradient should be such that no sparks pass, but that a faint blue glow is seen at each point, indicating the space in which most of the ionisation is taking place. The glow at the negative point is larger than that at the positive, and begins at a lower pressure. In either case ions of the same sign as that of the point are repelled, causing the electric wind. This was, with the pressures used in the present case, perceptible on the palm of the hand at a distance of about 10 cm.

The discharge can take place into free air, and the circuital flow is then completed either by diffusion of the ions to oppositely charged surfaces which are under the influence of the electrostatic machine used, or by recombination in the air. The latter is known to occur very rapidly in certain cases. In order, therefore, to expose the germs to a continuous and even wind for long periods, it was necessary to keep the machine running, and at the same speed, for the whole time of exposure.

3. The first arrangement of discharging points, that used in obtaining the results in figs. 7 to 10 (Plates 7 and 8), is shown in fig. 1. Two bell-jars,

* "On the Opposite Electrification produced by Animal and Vegetable Life," 'Roy. Soc. Proc.,' B, 1910, vol. 82.

5 inches diameter and 10 inches long, with stoppered openings at the top, were supported on ebonite and glass insulators. The discharging points, three fine steel sewing needles, were attached to a copper wire passing through the stopper to an electrostatic machine, one of the jars being connected in this way to the positive terminal, the other to the negative. The opposite pole in each case was connected by a wire entering through a central hole in the base plate to a thin metal disc, upon which was placed a Petrie dish containing agar sown upon the surface with an emulsion in water of the organism to be exposed. The distance of the points from the agar was in no case less than a centimetre.

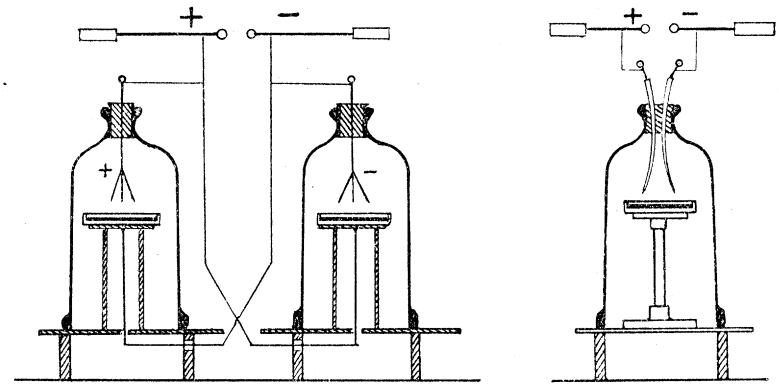


Fig. 1 Fig. 2

Fig. 1.—Discharging points in separate vessels.
Fig. 2.—Discharging points over the same plate.

The glow at the points could just be seen in diffused daylight. The photograph of fig. 3 (see at bottom of Plate 12), taken with the needles illuminated by a Nernst projector lamp, shows the proportions of the group and the volume of the negative glow in each case. From the photo-micrograph (fig. 4) the negative glow starts as a regular cone, drawn here to one side by the proximity of the positive pole, and the positive glow (fig. 5) appears on the side of the needle, in this case sharpened by a file cut. The former has a vertical angle of 135°, converging upon a point about 0·15 mm. outside of the surface of the needle.

The radial length of the glow is 0·10 mm., and its volume about 0·90 cu. mm. The positive glow is irregular and extended over a larger area on the needle than the negative, though the luminous volume is only about one-half that of the latter. With the arrangement shown in fig. 2 the current was 4·4 micro-ampères in both the positive and negative leads. At the negative pole the rate of ionisation, assumed to all take place in the

glow, is from the above figures 3×10^{13} charges, each of the magnitude e ($4{\cdot}6 \times 10^{-10}$ E.S.U.), per cubic millimetre per second. The photograph (fig. 6) shows a faint luminous line proceeding straight outwards from each of the positive points, distinct from the ordinary visible glow. This is only found after long exposure—three-quarters of an hour—of the photographic plate. It is possible that this is the space in which negative ions are produced and receive inward acceleration in positive point discharge. Some of these would be, no doubt, carried away by the positive wind, and this might account for the presence of negative ions in positive point discharge observed by C. T. R. Wilson and by N. R. Campbell. The luminosity may also be from ionisation produced by "Entladungstrahlen."* No such line is to be seen at the negative poles. The length is here 8 mm. At both poles there is a bright luminous patch on the surface of the metal, but at the positive point there is also some scintillation, possibly due to the negative bombardment, which is not seen at the negative point. The voltage used was not directly measured, but gave sparks 2 to 2·5 mm. long between 1·5 cm. diameter brass discharge knobs. On open circuit, that is, with the needle points disconnected, the machine gave 1·5 cm. sparks, running at the same speed as before.

4. The second arrangement of discharging points, that used in obtaining figs. 11 to 15 (Plates 9 and 10), differed only from the first in having both positive and negative points brought into the same jar, as in fig. 2. The needle points emerged 2 mm. from the leading-in glass tubes, and were directed to positions on the plates $2\frac{1}{2}$ cm. centre to centre. The stand holding the exposed plate was supported by a sulphur rod, attached to an ebonite base-plate. The leak to earth other than from point to point was exceedingly small. The object of having both points over the same culture was to obtain comparison of exposures in which the current passing was the same for both. The outer part of the agar surface provided a control, which for short exposures may be taken as unexposed, though in certain cases with prolonged exposure the whole plate is cleared. With the two points discharging on to the same surface the action was much stronger than in the first arrangement.

5. Of the organisms exposed in the first way (fig. 1) photographs of typical exposures are given of *B. anthracis*, *B. pyocyaneus*, *Sarcina lutea*, *Pneumococcus*; and in the second (fig. 2) of *B. coli communis*, *B. Friedländer*, *B. typhosus*, *B. asiaticæ choleræ*, *B. dysenterica Shiga*. These were all fresh, active growths, though most of them had been often sub-cultured.

It was found that there was a marked difference between the sensitiveness

* *Vide* Sir J. J. Thomson, "Conduction of Electricity through Gases," §§ 242 and 310.

of the bacteria examined. On account of the use made by previous experimenters of *B. anthracis*, *B. typhosus*, and *B. coli communis*, these were used in determining the best conditions of exposure.

In fig. 7 (Plate 7) the organism is anthrax exposed for 20 minutes, 10 minutes' exposure failing to show any effect. The colonies in the control are large and uniformly distributed. In the central (positive) plate they are cleared opposite two points, the third has missed fire. In the negative plate the cleared areas are somewhat larger and the colonies fewer. In fig. 8 the exposure was for 30 minutes, and the influence of time and of difference of sign of charge is now unmistakable. The positive plate is well cleared, the negative contains one colony only, and at a corner where the wind is least likely to have taken effect. In fig. 9 (Plate 8) with 50 minutes' exposure of a dense sowing the positive plate shows little growth, and on the negative there is one colony. Many hours' exposure are necessary to completely sterilise the plates with certainty in this way. Fig. 10 is of Pneumococcus, 1 hour 10 minutes' exposure. From a very dense sowing only about a dozen colonies developed on the positive plate and a few rudimentary ones on the negative.

In the case of *B. pyocyaneus*, exposed as in fig. 1, the negative plate was entirely cleared in two and a-half hours, the positive about one-half. The control showed a dense growth. This may be regarded as a sensitive organism, though not to the same degree as *B. asiaticæ choleræ*, given later.

Sarcina lutea, exposed for two and a-half hours, gave nearly as many colonies on the positive plate as on the control, the negative having only a few small ones.

6. On account partly of the long time sometimes taken to produce a distinctive difference, the arrangement was changed to that shown at fig. 2. The first exposure of *B. coli communis* was for 18 hours. On examining the dish it was found that the discharge had dried the jelly, forming two shallow pits, the smaller caused by the positive discharge, the larger by the negative. The angle of the positive cone was one-half that of the negative.

The *B. coli communis* series is given in fig. 11 (Plate 9), fig. 13 (Plate 10), and fig. 18 (Plate 11), the exposures in Plate 9 being $1\frac{1}{4}$, $4\frac{1}{2}$, and 18 hours respectively. A good deal is cleared in the first, all but a small patch in the bottom corner of the second, and the third entirely so. Fig. 12 is of *B. typhosus*, the times of exposure being 1 hour, $\frac{1}{2}$ hour, and $2\frac{1}{4}$ hours. There is a trace of clearing in the $\frac{1}{2}$-hour plate, none at the positive in 1 hour, though a good deal at the negative, and in $2\frac{1}{4}$ hours an almost clear plate.

The two upper photographs, fig. 13 (Plate 10) are of *B. coli communis*, the right hand exposed for 1 hour, the left for ½ hour, in the latter of which the signs of clearing are slight.

The organism most sensitive to the point discharge of any so far examined is *B. asiaticæ choleræ*, two exposures of which are given in fig. 14 (Plate 10), that to the left having been exposed for a quarter of an hour, that to the right for half an hour. This bacterium is also sensitive to light, dying out in a few days in diffused daylight.

In the case of *B. dysenterica Shiga*, fig. 15 (Plate 10), half an hour had little or no effect, the whole surface being covered with fine densely packed colonies. In an hour, however, most of the plate was cleared. This and the above result with *B. asiaticæ choleræ* may be compared with the observation of Buchner, that the growth of *B. typhosus* is prevented by exposing a freshly-sown culture for an hour to full sunlight.

From the above results it may be concluded that: (1) air ionised by either positive or negative point discharge has a strong bactericidal action; (2) the negative discharge is much more effective than the positive for short exposures, though the result after many hours' exposure is nearly the same for both. It is possible that some part of the bactericidal action of the positive wind is owing to negative ions produced at the positive point.

7. Since oxygen is electro-negative and ozone is known to be produced by electrical discharge, the electric wind was examined for ozone by paper which had been moistened with a solution in alcohol of tetra-methyl-*p.p.*-diamido-diphenyl-methane.* This has the property of turning violet in the presence ozone, yellow with nitric oxide. On exposure to the discharge arranged as in fig. 2, it was found that ozone was produced at the negative point and to a much less degree at the positive. There was no yellow coloration, though the paper dried a purplish brown. The presence of ozone in the wind suggested the possibility that the bactericidal action might only be indirectly the result of ionisation, that the well-known sterilising influence of ozone (whatever the ultimate cause of that may be) might explain the facts equally well. To decide this, exposures were made in nitrogen and pure hydrogen. The former was, however, found to contain 0·8 per cent. of oxygen, and since nitrogen when mixed with oxygen even in small quantities cannot be regarded as quite inert, the result given in fig. 20, obtained in it using *B. typhosus*, is chiefly of interest in showing that the effect is of the same order of magnitude as in hydrogen and air. In the case of hydrogen the bell-jars were well exhausted and filled with the gas several times before exposure, with the

* See Fischer and Braemar, "On the Production of Ozone by Ultra-violet Light," 'Ber.,' 1905, vol. 38, No. 3, p. 2633.

Petrie dishes in position containing the bacteria. The photographs of Plate 11 of *B. asiaticæ cholerœ*, fig. 16; *B. typhosus*, fig. 17; and *B. coli communis*, fig. 18; in hydrogen, with the discharging point arranged as in fig. 19, show that the sterilising influence of the negative discharge is somewhat greater in hydrogen at atmospheric pressure than in nitrogen or, by comparison with the previous results, than in air, owing possibly to the greater velocity and range of ions in hydrogen. It would, however, appear that in these cases the nature of the gas is not of the first importance, and that it is the presence of the electrical charge which is the chief inhibiting cause.

The criticism having been made that water-vapour from the surface of the agar might give rise to the formation of hydrogen peroxide in the glow, trials

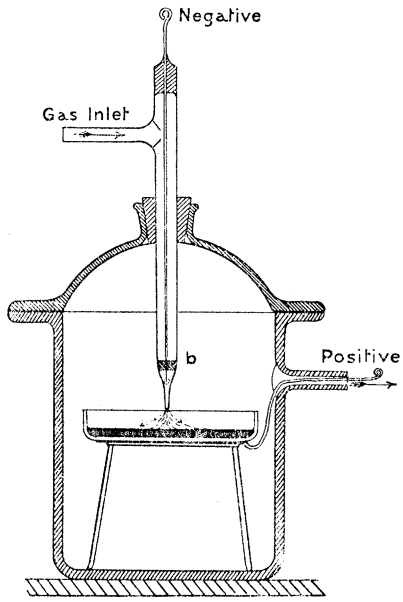


Fig. 19.—Discharge point in tube.

were made with the exposure vessel arranged as in fig. 19. The wire to which the discharging needle is soldered is led down a glass tube through which a stream of the gas to be used is slowly passed. The needle is held in a central position by a glass bead *b*, and the gas, ionised by the discharge, is blown gently upon the agar surface sown with bacteria. The Petrie dish rests on a metal plate connected to the positive pole. There is no possibility of water-vapour reaching the needle point, and the arrangement is very convenient for the examination of the effect of ionised gases on surface cultures. In operation the outlet from the jar was first sealed with paraffin wax and the

vessel exhausted and filled several times with hydrogen passed through sulphuric acid and tubes of soda-lime. An opening was then made by passing a hot wire through the wax, and a continuous stream of gas sent past the electrified needle from a cylinder of hydrogen. Fig. 18 (Plate 11) shows the result of exposing plates of Conradi and Drigalski litmus lactose medium sown with *B. coli communis*, which were before exposure inverted and dried in a warm chamber to fix the emulsion on the surface. The jet of hydrogen without ionisation was passed for three-quarters of an hour over one plate, which was then removed. The electrostatic machine was then started and the other plate exposed for the same time to the same stream of hydrogen, now negatively ionised. The influence of the electrification is marked, the area beneath the nozzle being quite cleared. This experiment is also of interest in showing that pure dry hydrogen has no inhibiting effect on bacterial growth. The conditions in this test were the most stringent that could be devised. The surface of the agar was firm and dry and the time of exposure short for so insensitive an organism as *B. coli communis*. The plate may be compared with fig. 13, where the exposures were for half and one hour using the same bacillus.

The exposures in nitrogen and hydrogen were made in the Pathological Laboratory of the University of Durham College of Medicine, under the direction of Professor Hutchens, to whom, with his colleague, Dr. P. Laws, the author is gratefully indebted.

8. When bacteria are killed by any physical or chemical agency it is generally accepted to be in consequence of the coagulation of protoplasm. In the present case there are two immediate possibilities, apart from direct electrical action, by which this might be achieved. The effect may be due to the influence of ultra-violet light from the glow, or the mechanical bombardment by the wind might be sufficient to produce inhibition of growth such as is known to be caused by mechanical vibration. To put the first to the test a piece of optical quartz, 2·4 cm. square by 1 mm. thick, was laid on the surface of the agar, which had previously been sown with a strong emulsion of *B. asiaticæ choleræ* as a sensitive indicator. The dish was then exposed for half an hour in such a position that the negative needle point was central with the quartz square and at 1 cm. above it. The positive point was then about 1·5 cm. from the edge of the square. At the end of the exposure the quartz was removed, care being taken to prevent liquid from the edges flooding into the space occupied, though some unavoidably ran over the space under the positive needle. The result on incubation is given on the right of fig. 21. The growth on the part covered by the quartz is denser than elsewhere, the space around showing evident signs of clearing.

It may then be concluded that the bactericidal effects observed in the photographs are not due to the influence of ultra-violet light, bactericidal rays of which would freely penetrate the thickness of air and quartz used.

In order next to shield the organisms from the direct wind by interposing a stout membrane between the point and the agar, a plate was prepared and sown upon which a cigarette paper was laid moistened with the emulsion. The discharging points were arranged to be on the centre line of the paper, and the exposure was, as before, half an hour. The effect, also given in fig. 21, shows that the negative discharge penetrates the moist paper, though it is not so active as without the paper. This result, that the destructive influence can penetrate a membrane like wet paper in contact with bacteria, is of importance in showing that the electrical charge of ionised air may be expected to pass through lung membranes into the blood.

There is, however, in the present case a third possibility, that the action is not caused by the ions carried with the electric wind but by some penetrating radiation independent of it. To test this a light wire frame was made to hold a cigarette paper, dry or moistened as before, 3 mm. horizontally above the surface of the agar, which had been sown as usual. The result was that there were no cleared areas under the points. The whole effect may therefore be attributed to the direct influence of, and contact with, ions in the electric wind.

9. It was shown in the previous paper that both red corpuscles and leucocytes have a strong negative charge. On the other hand, fresh bacteria have a charge positive in sign and of the same order mass for mass as that of the blood cells.

The chief function of leucocytes is known to be that of absorbing bacteria from the blood. Their thin walls are easily pierced, but the origin of the force necessary for this to take place has not been located. The general conception of chemiotaxis, covering all movement in response to chemical stimulus, has been the closest approximation. It is now suggested that the initial stimulus to the process of absorption may be more simply explained by the attraction of the positively charged bacteria by the large negatively charged leucocytes. In the case of fresh blood cells the electrical charges are very fixed and characteristic, as shown by the rapid and uniform movement of the cells in strong electric fields. The charge of bacteria, however, invariably reverses when the culture is kept for several days.* The negative chemiotaxis described by Bordet† would then have to be

* 'Roy. Soc. Proc.,' B, 1910, vol. 82, p. 641.

† J. Bordet, "Studies on the Serum of Vaccinated Animals," 'Annales de la Société Royale des Sciences médicales et nat. de Bruxelles,' 1895, vol. 4.

interpreted by some such reversal occurring normally in the infected animal, on account of changes in the blood serum or peritoneal fluid.

DESCRIPTION OF PLATES.

Plate 7.

Fig. 7.—*B. anthracis.* 20 minutes' exposure.
Fig. 8.—*B. anthracis.* 30 minutes' exposure.

Plate 8.

Fig. 9.—*B. anthracis.* 50 minutes' exposure.
Fig. 10.—*Pneumococcus.* 70 minutes' exposure.

Plate 9.

Fig. 11.—*B. coli communis.* $1\frac{1}{4}$, $4\frac{1}{2}$, and 18 hours' exposure.
Fig. 12.—*B. typhosus.* 1 hour and $2\frac{1}{4}$ hours' exposure.

Plate 10.

Fig. 13.—*B. coli communis.* $\frac{1}{2}$ hour and 1 hour's exposure.
Fig. 14.—*B. asiaticæ choleræ.* $\frac{1}{4}$ hour and $\frac{1}{2}$ hour's exposure.
Fig. 15.—*B. dysenterica Shiga.* $\frac{1}{2}$ hour and 1 hour's exposure.

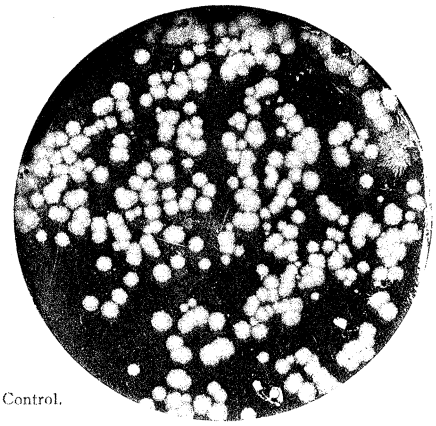
Plate 11.

Fig. 16.—*B. asiaticæ choleræ* in hydrogen. 1 hour's exposure.
Fig. 17.—*B. typhosus* in hydrogen. 1 hour's exposure.
Fig. 18.—*B. coli communis* in hydrogen. 1 hour's exposure.

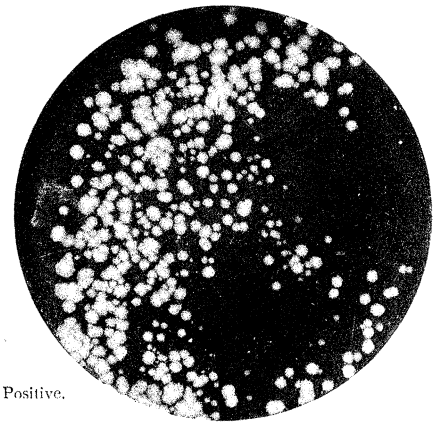
Plate 12.

Figs. 3, 4, 5.—Point discharge.
Fig. 6.—Streamers in positive point discharge.
Fig. 20.—*B. typhosus* in nitrogen. Top.
Fig. 21.—*B. asiaticæ choleræ* under quartz and paper. Bottom.

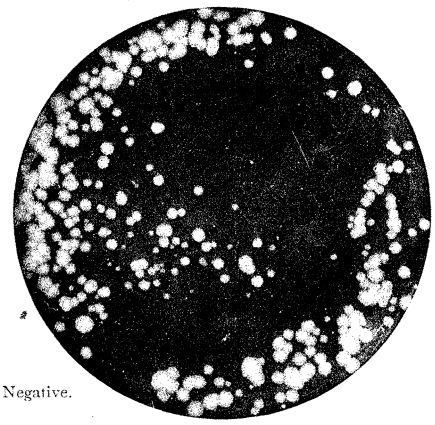
FIG. 7.
B. Anthracis, 20 mins.



Control.

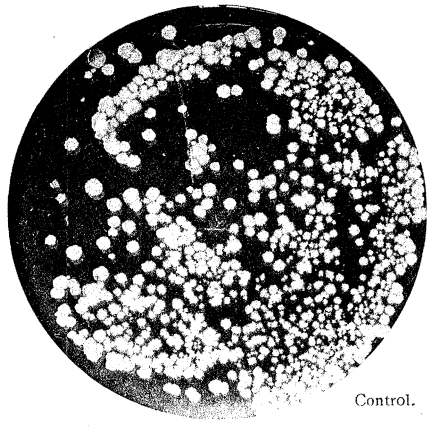


Positive.



Negative.

FIG. 8.
B. Anthracis, 30 mins.



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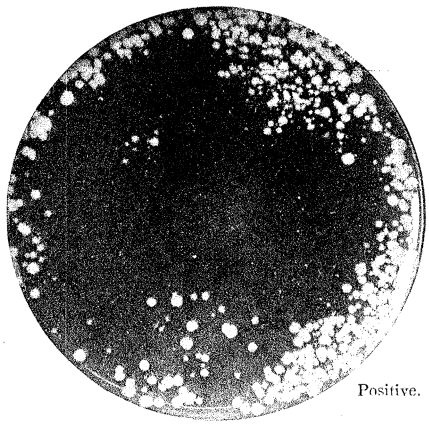
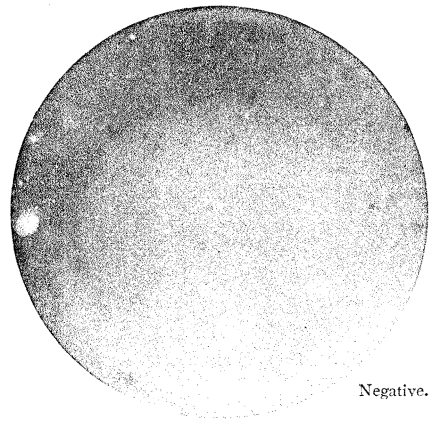
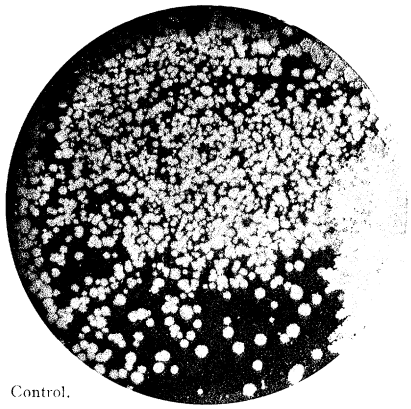


Positive.



Negative.

FIG. 9.
B. Anthracis, 50 mins.



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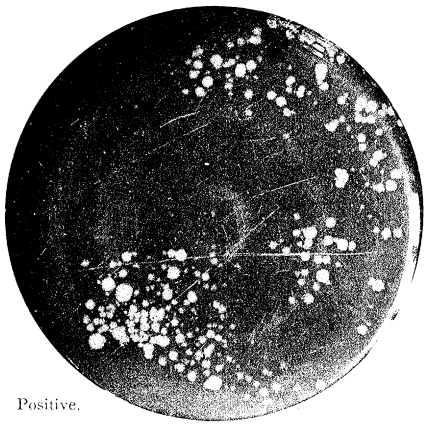
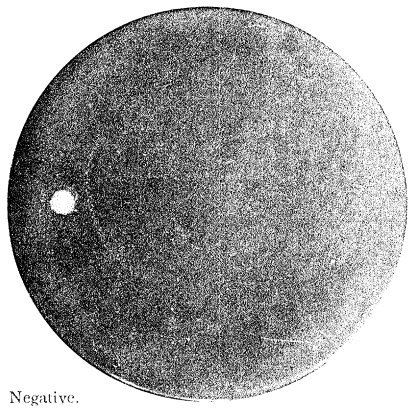
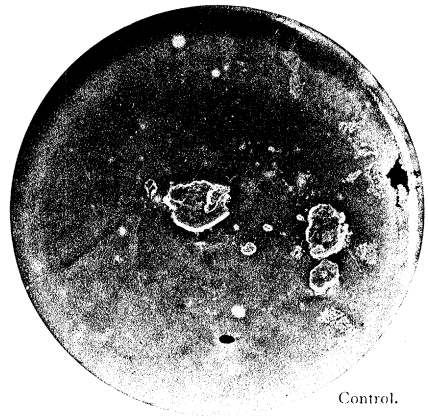


Positive.

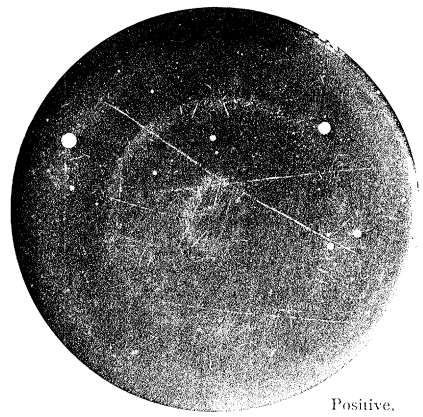


Negative.

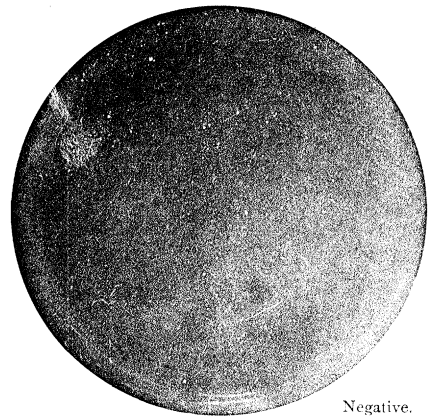
FIG. 10.
Pneumococcus, 70 mins.



Control.

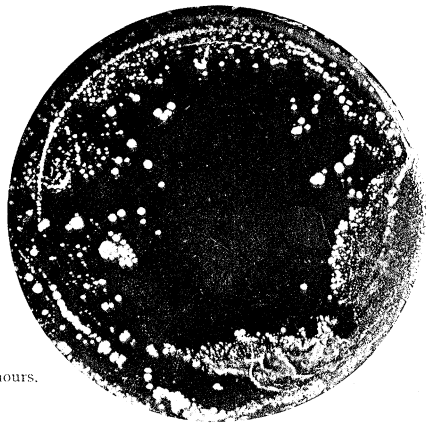


Positive.



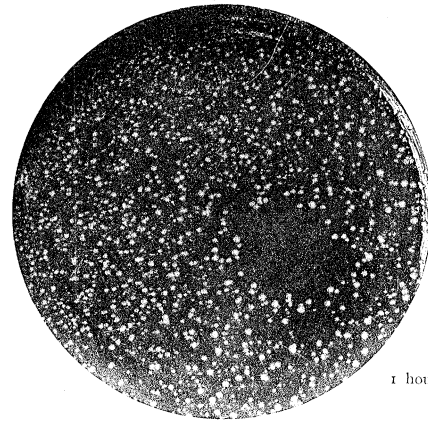
Negative.

FIG. 11.
B. Coli Communis.

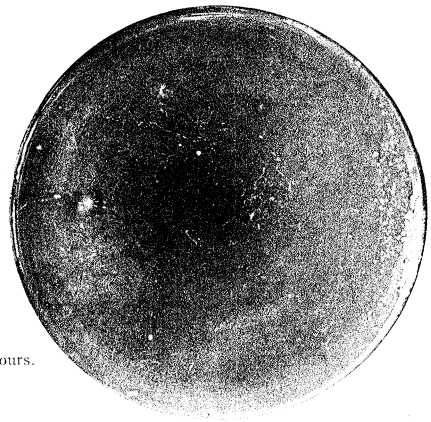


$1\frac{1}{4}$ hours.

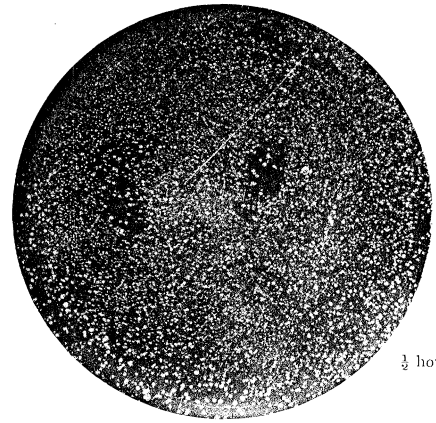
FIG. 12.
B. Typhosus.



1 hour.

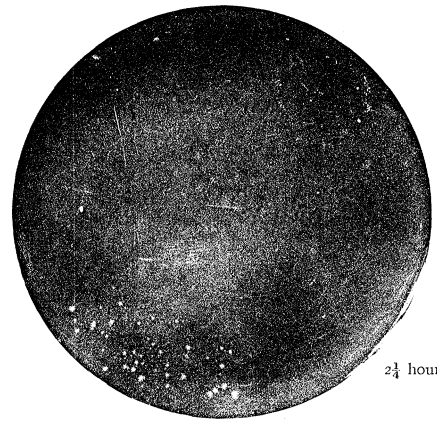


$4\frac{1}{2}$ hours.



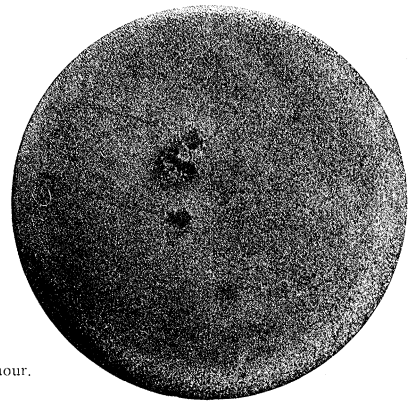
$\frac{1}{2}$ hour.

18 hours.



$2\frac{1}{4}$ hours.

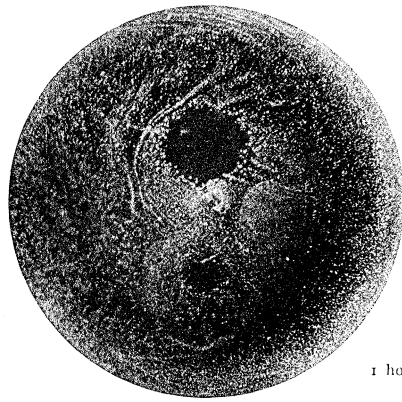
Exposed as in Plate 8.



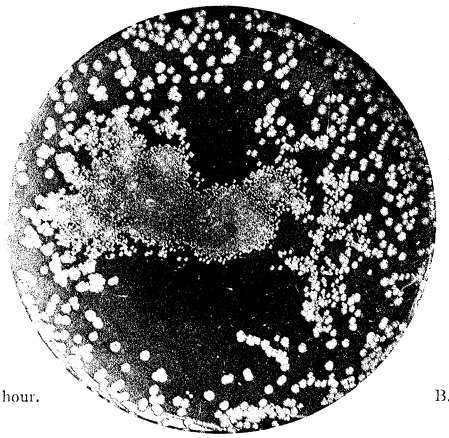
½ hour.

FIG. 13.

B. Coli Communis.



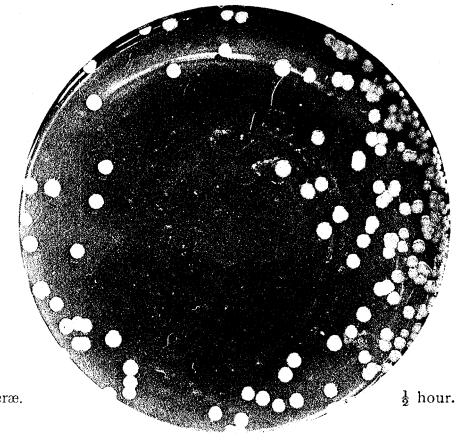
1 hour.



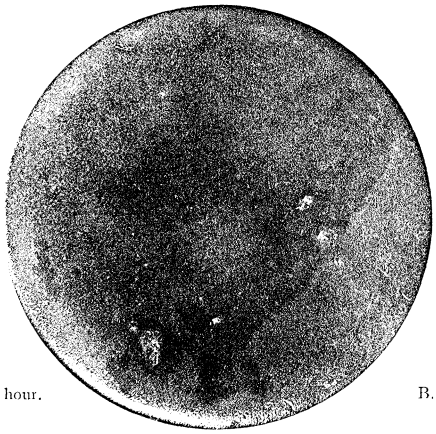
¼ hour.

FIG. 14.

B. Asiat. Choleræ.



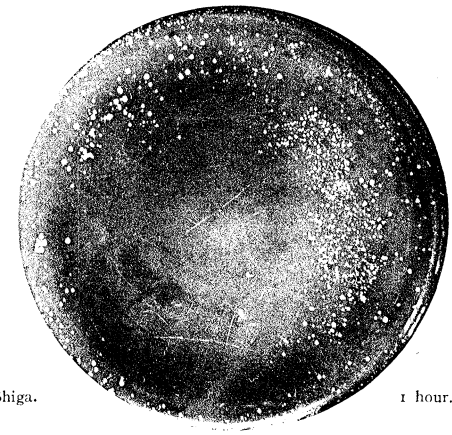
½ hour.



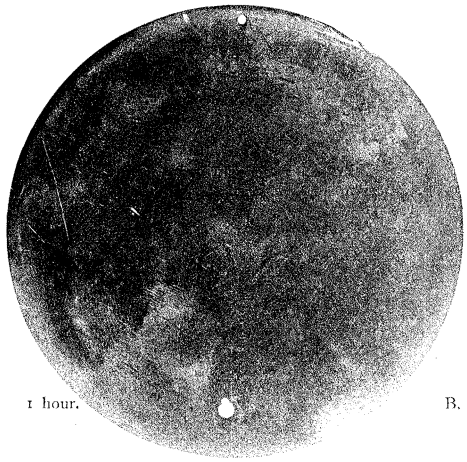
½ hour.

FIG. 15.

B. Dysenterica, Shiga.



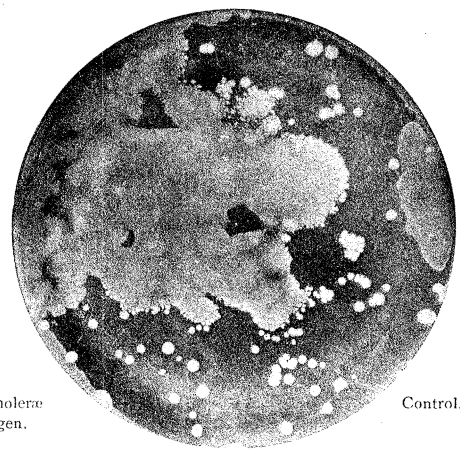
1 hour.



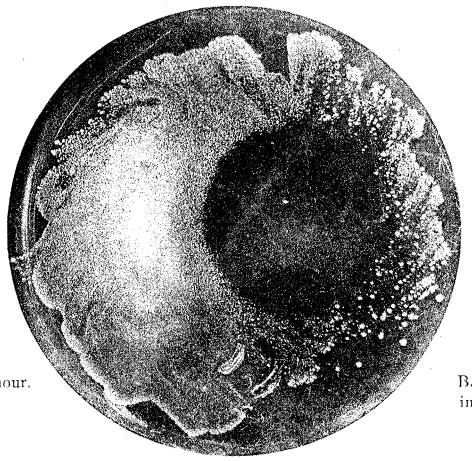
1 hour.

FIG. 16.

B. Asiat. Choleræ in hydrogen.



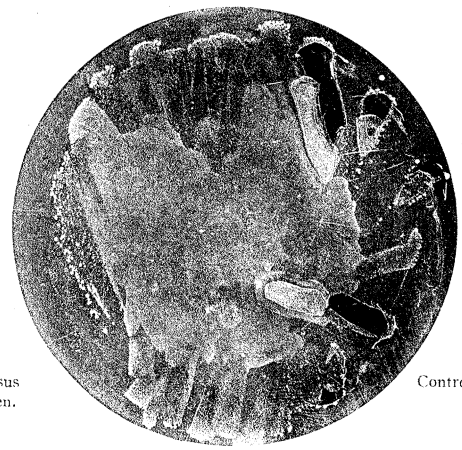
Control.



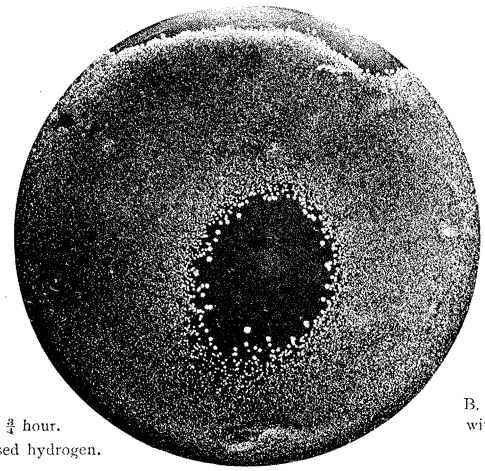
1 hour.

FIG. 17.

B. Typhosus in hydrogen.



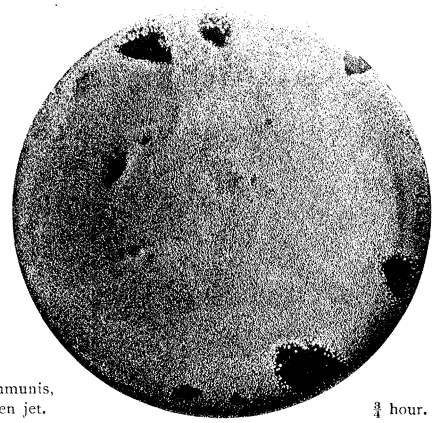
Control.



$\frac{3}{4}$ hour.
Ionised hydrogen.

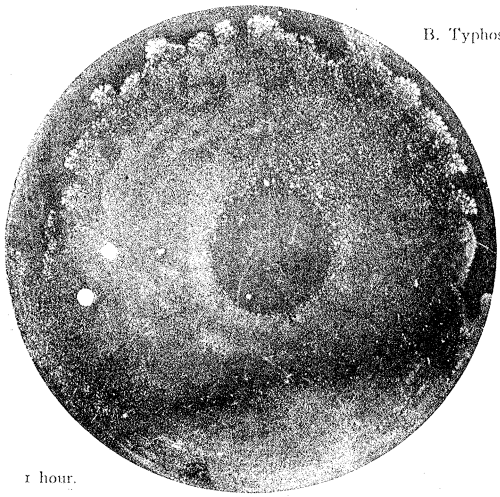
FIG. 18.

B. Coli Communis, with hydrogen jet.

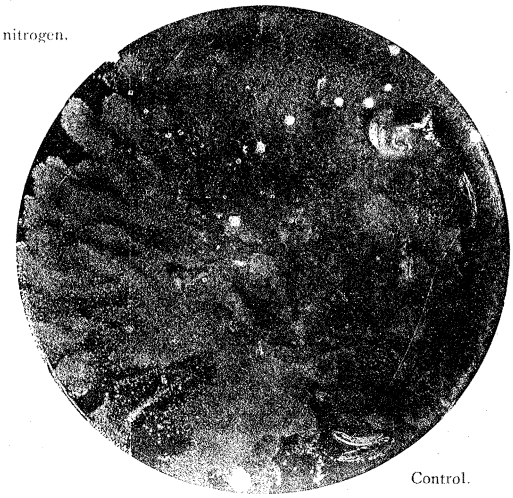


$\frac{3}{4}$ hour.
Hydrogen alone, no effect.

B. Typhosus in nitrogen.



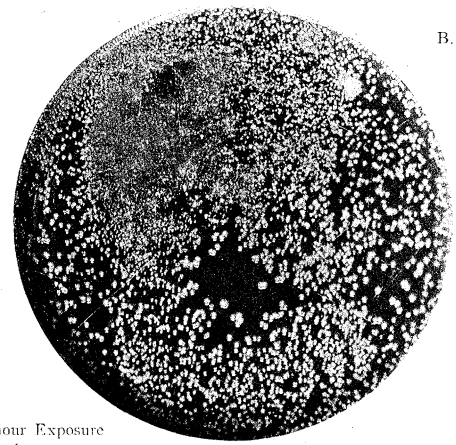
1 hour.



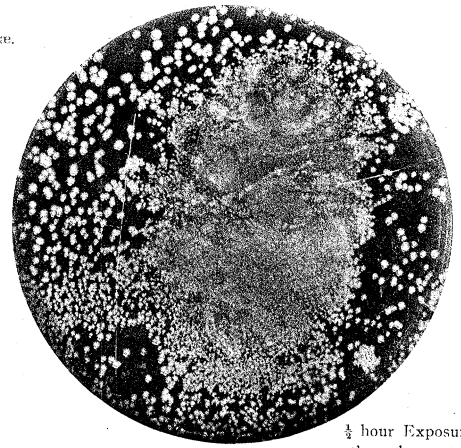
Control.

FIG. 20.

B. Asiat. Choleræ.



¼ hour Exposure through wet paper.



¼ hour Exposure through quartz.

FIG. 21.

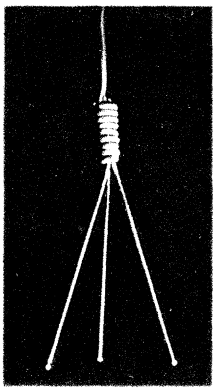


FIG. 3.

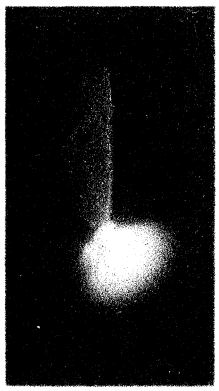


FIG. 4.

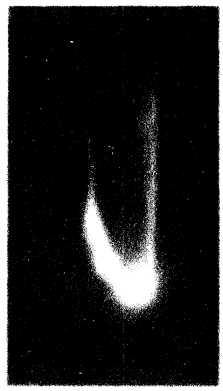


FIG. 5.

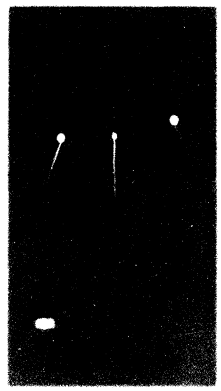
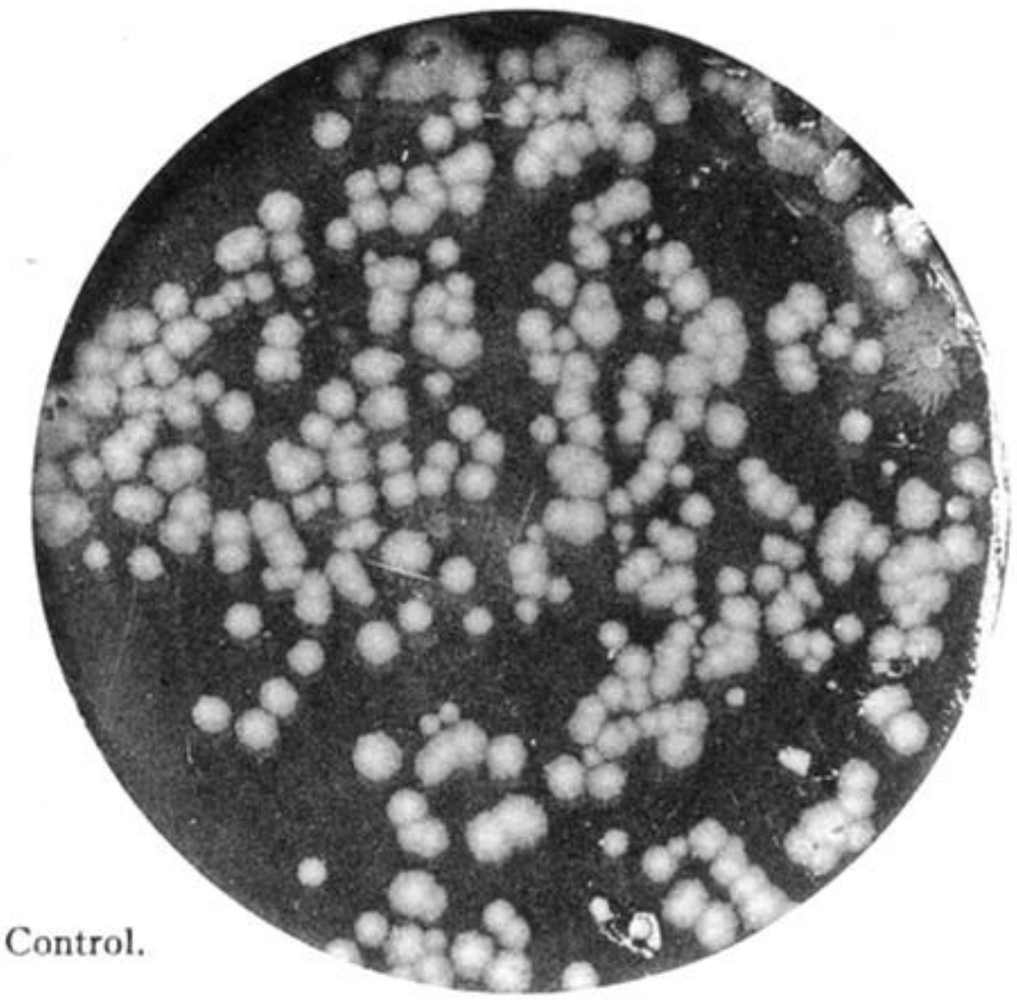


FIG. 6.

FIG. 7.

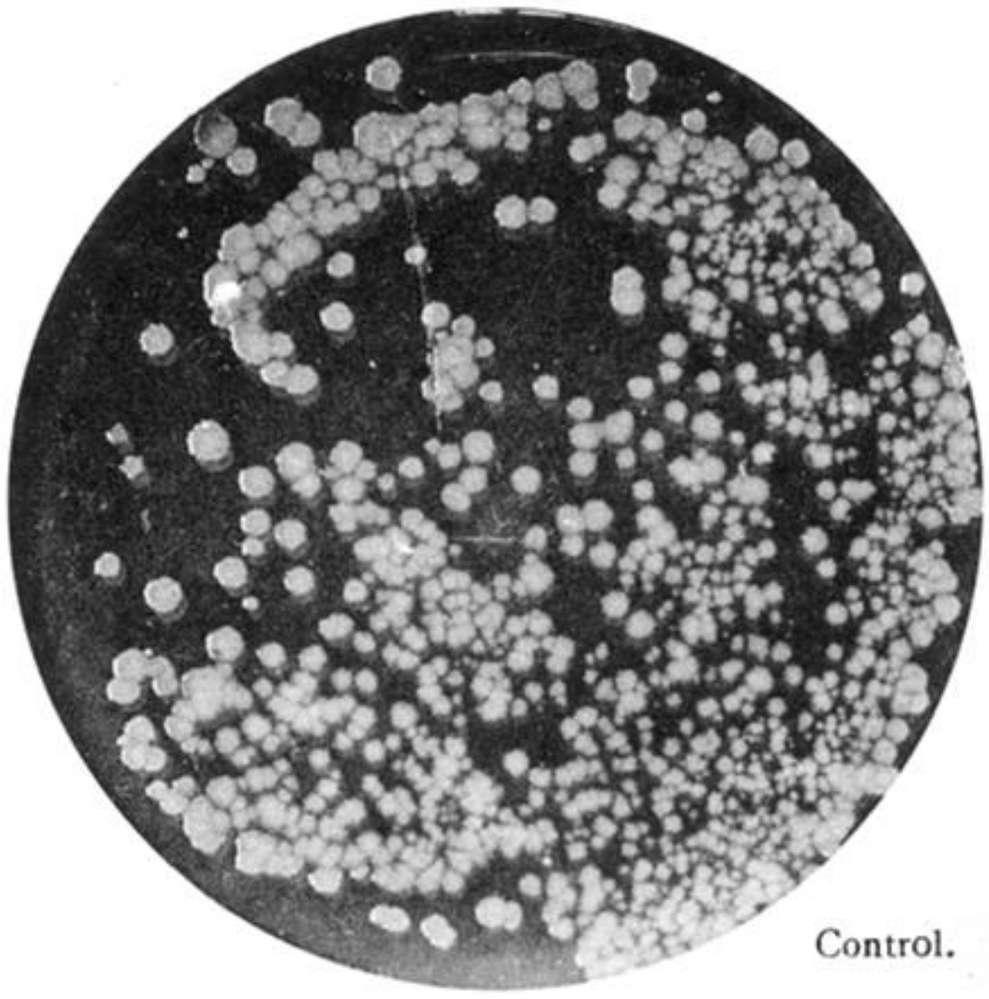
B. Anthracis, 20 mins.



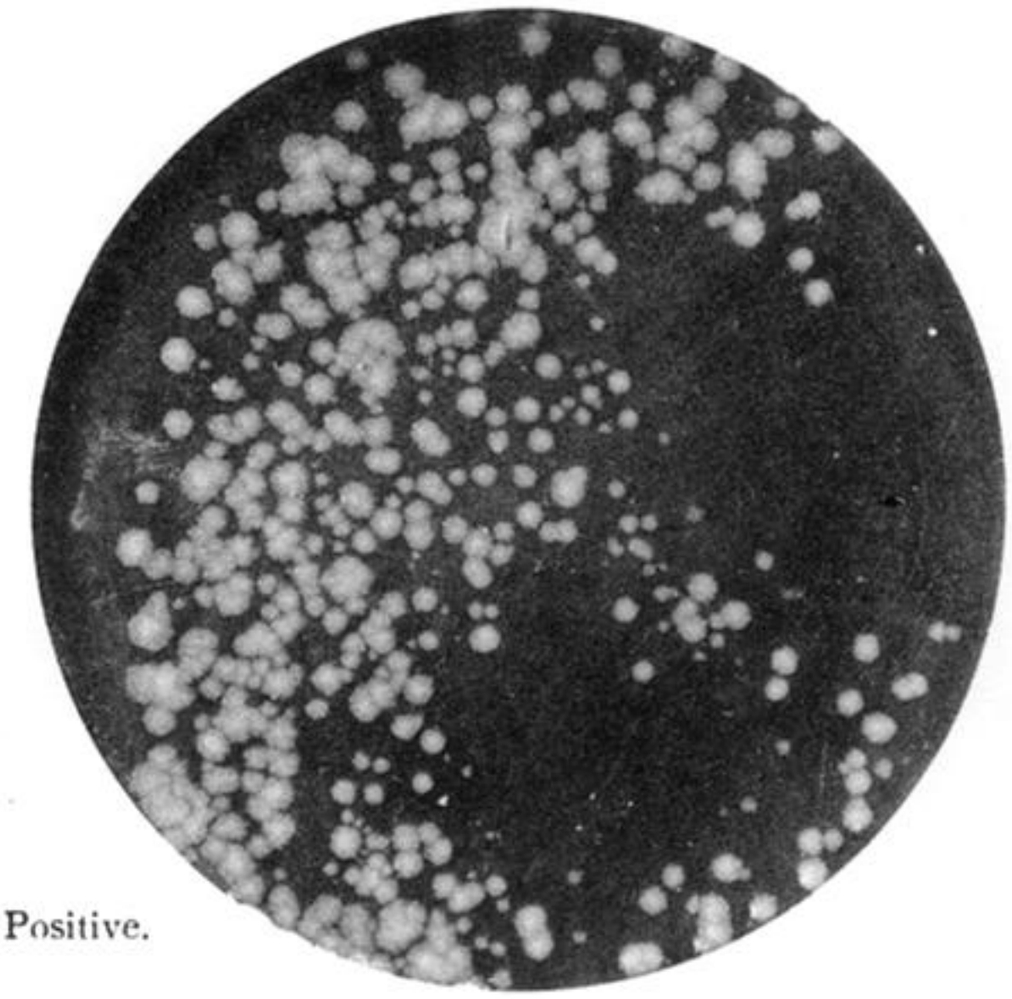
Control.

FIG. 8.

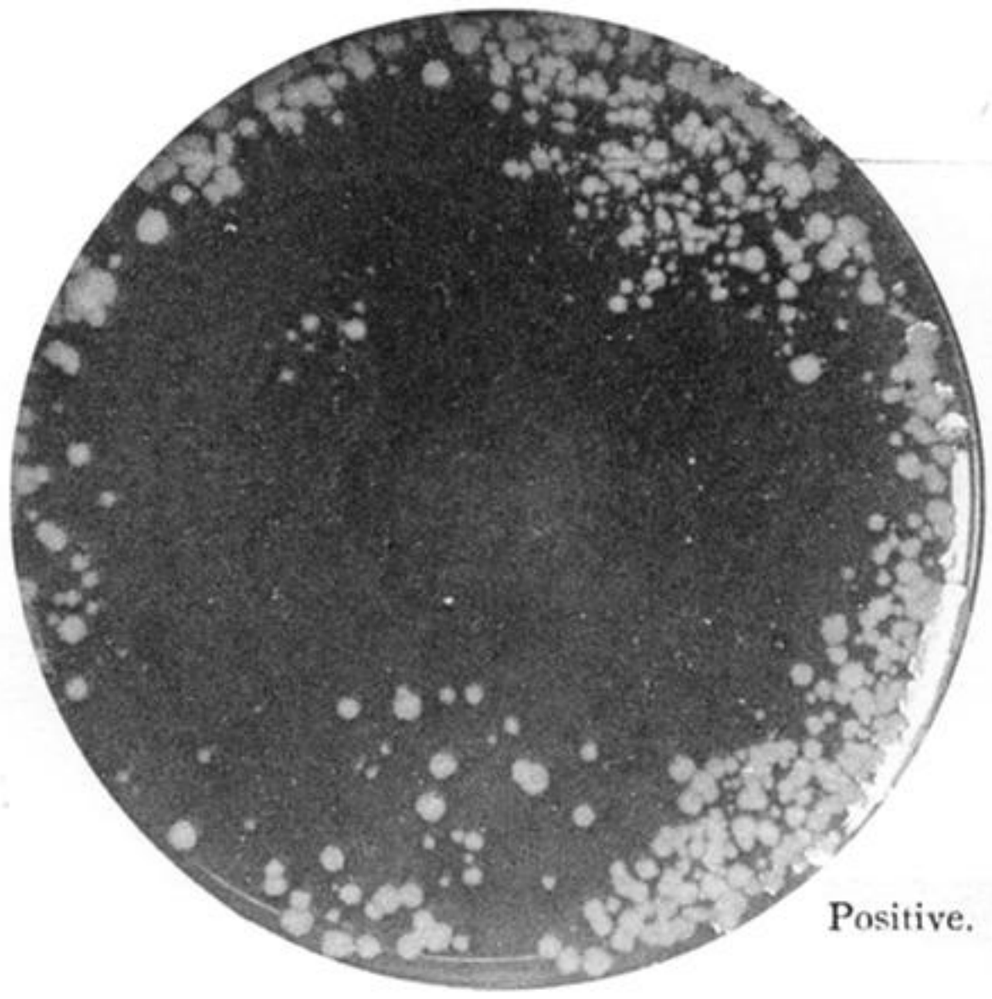
B. Anthracis, 30 mins.



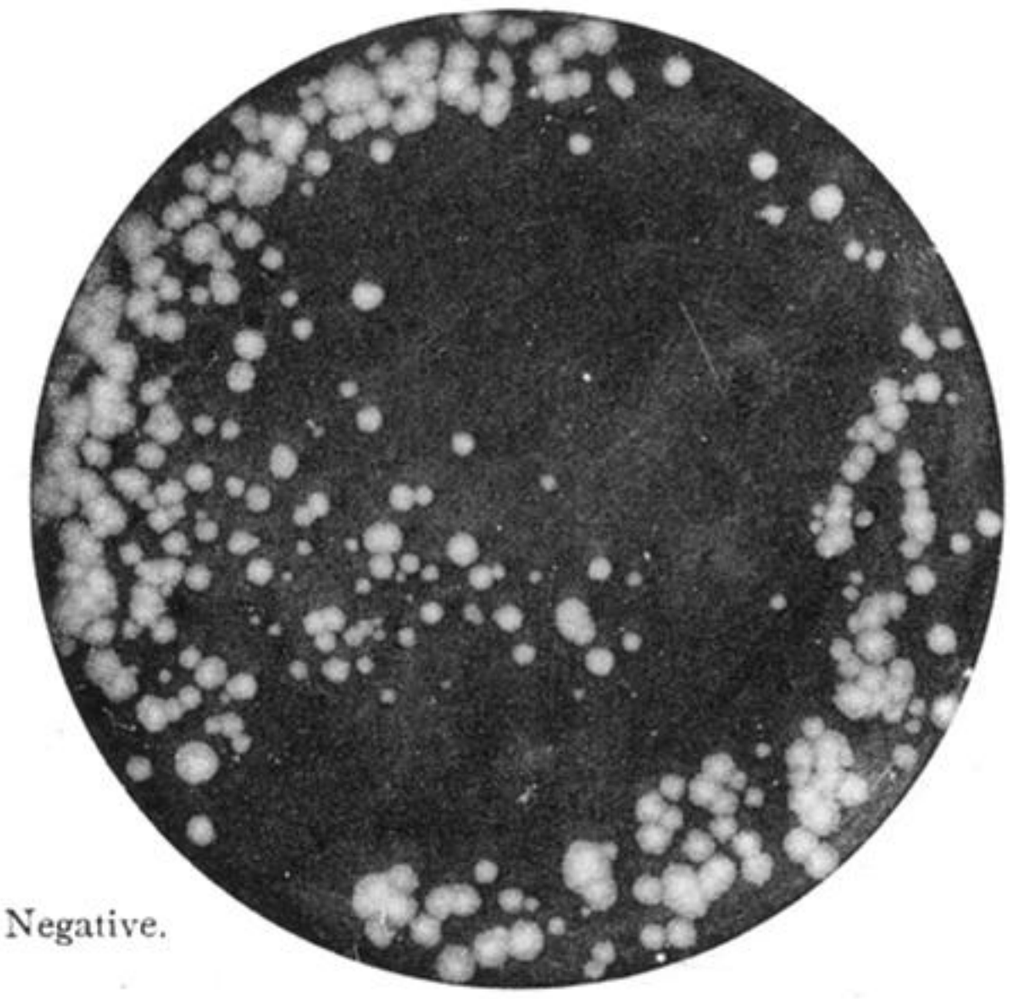
Control.



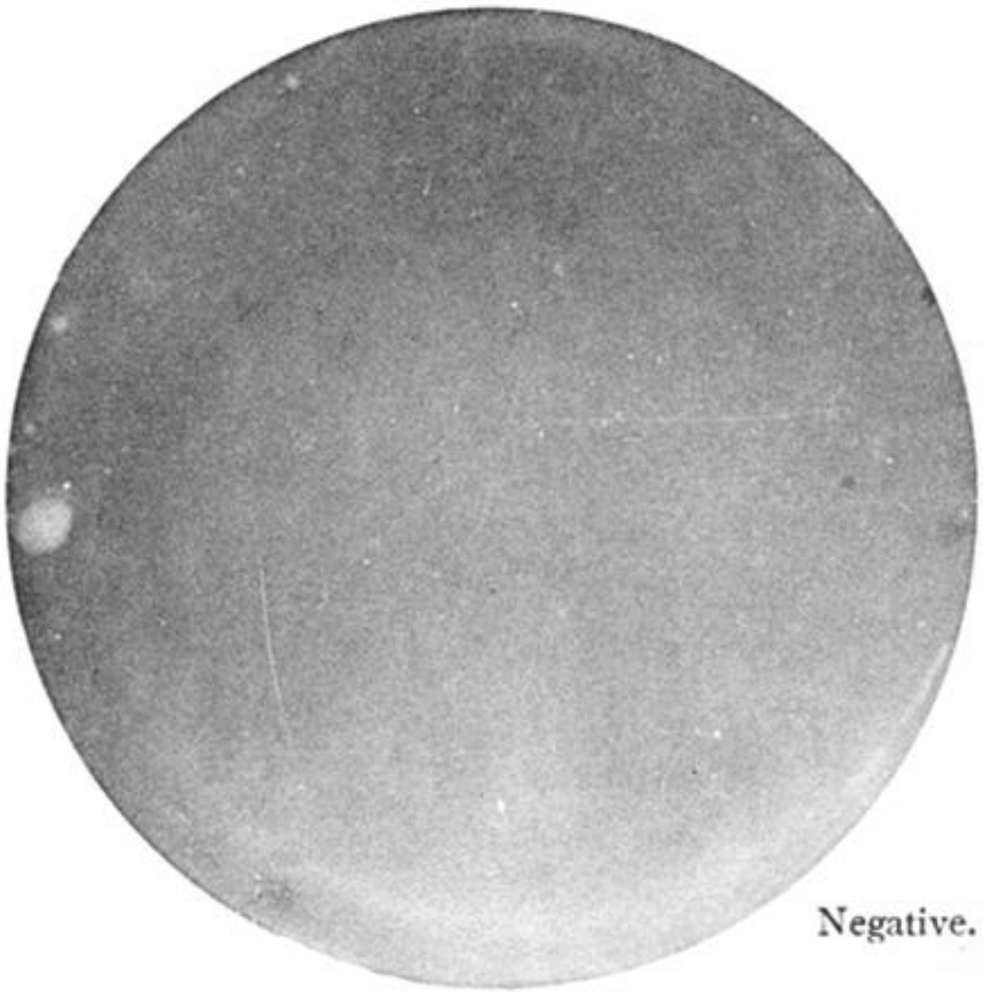
Positive.



Positive.

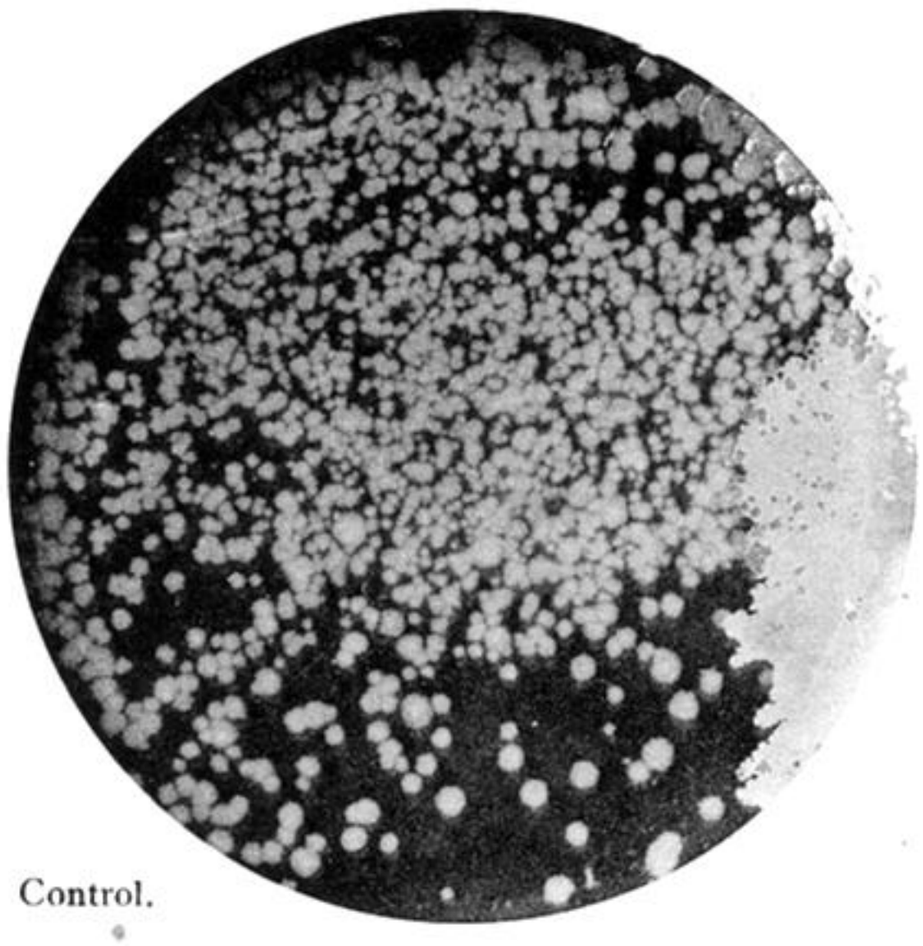


Negative.

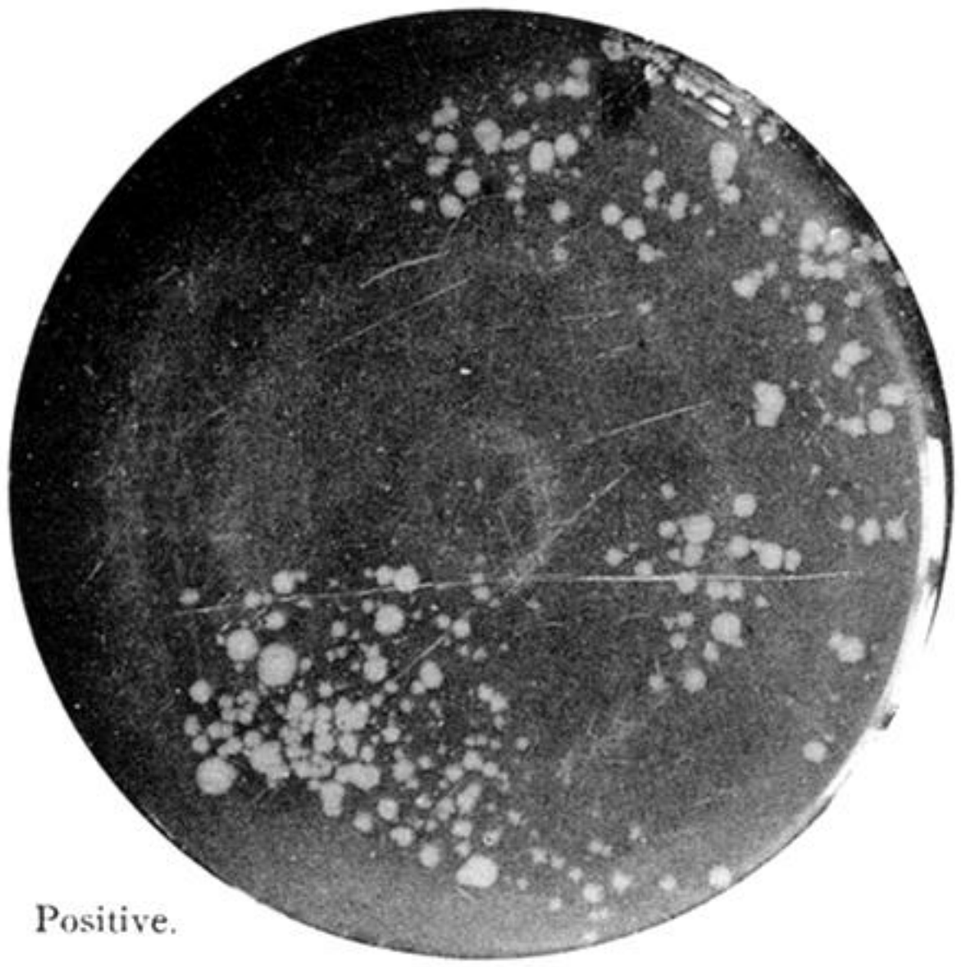


Negative.

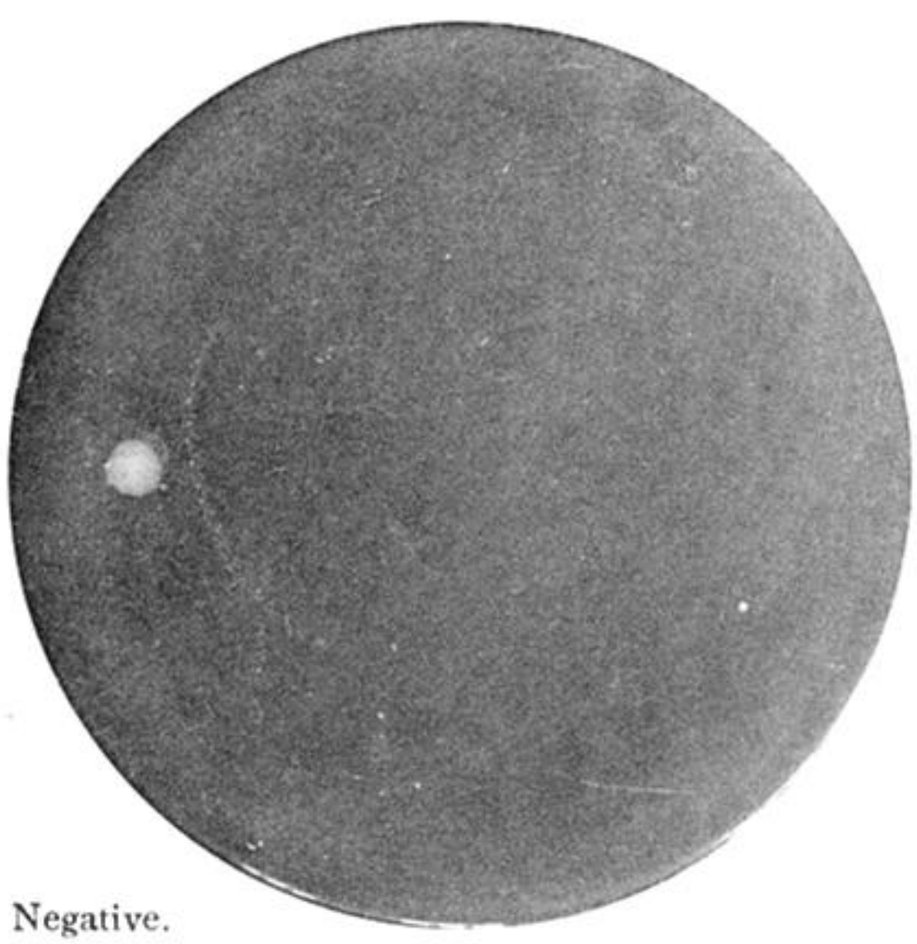
FIG. 9.
B. Anthracis, 50 mins.



Control.

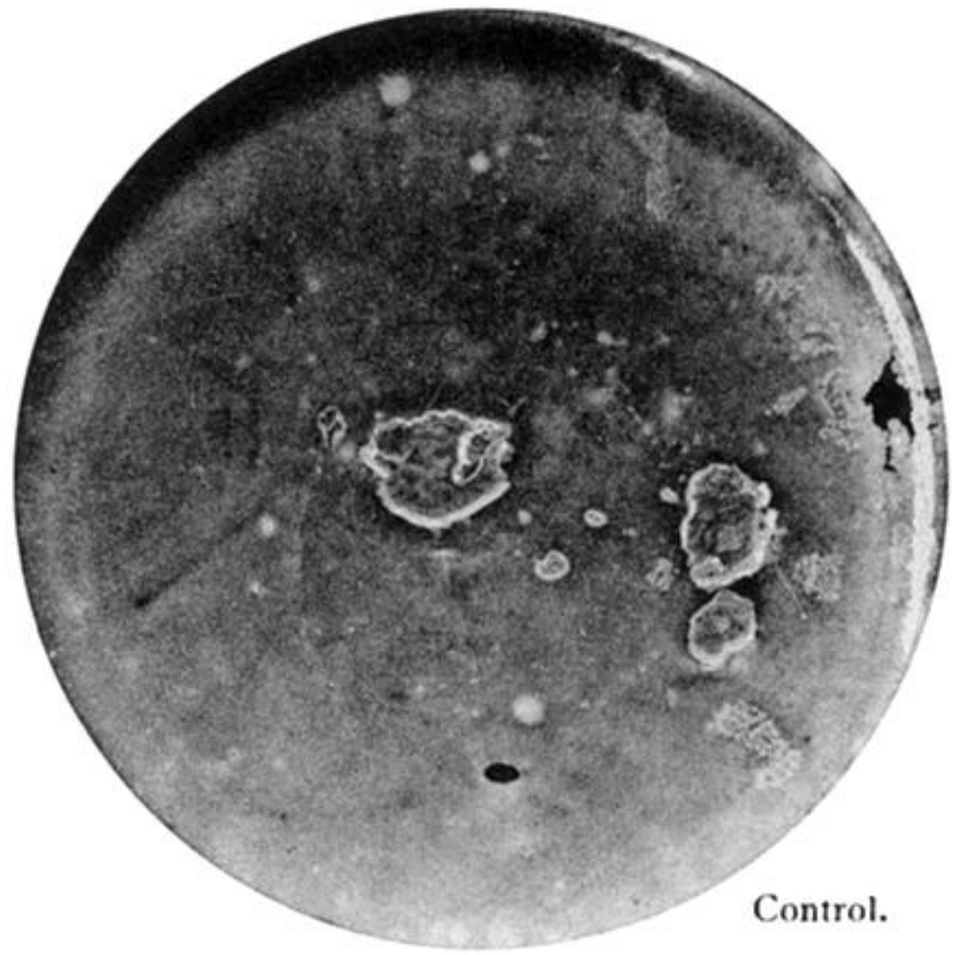


Positive.

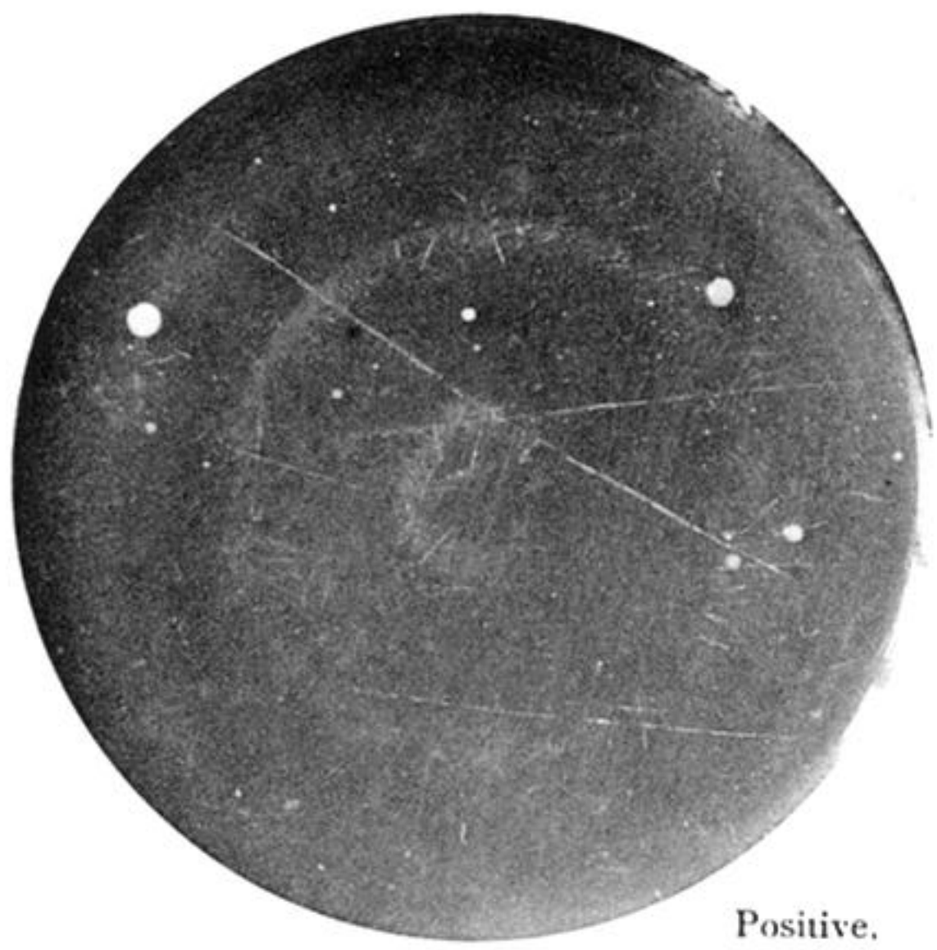


Negative.

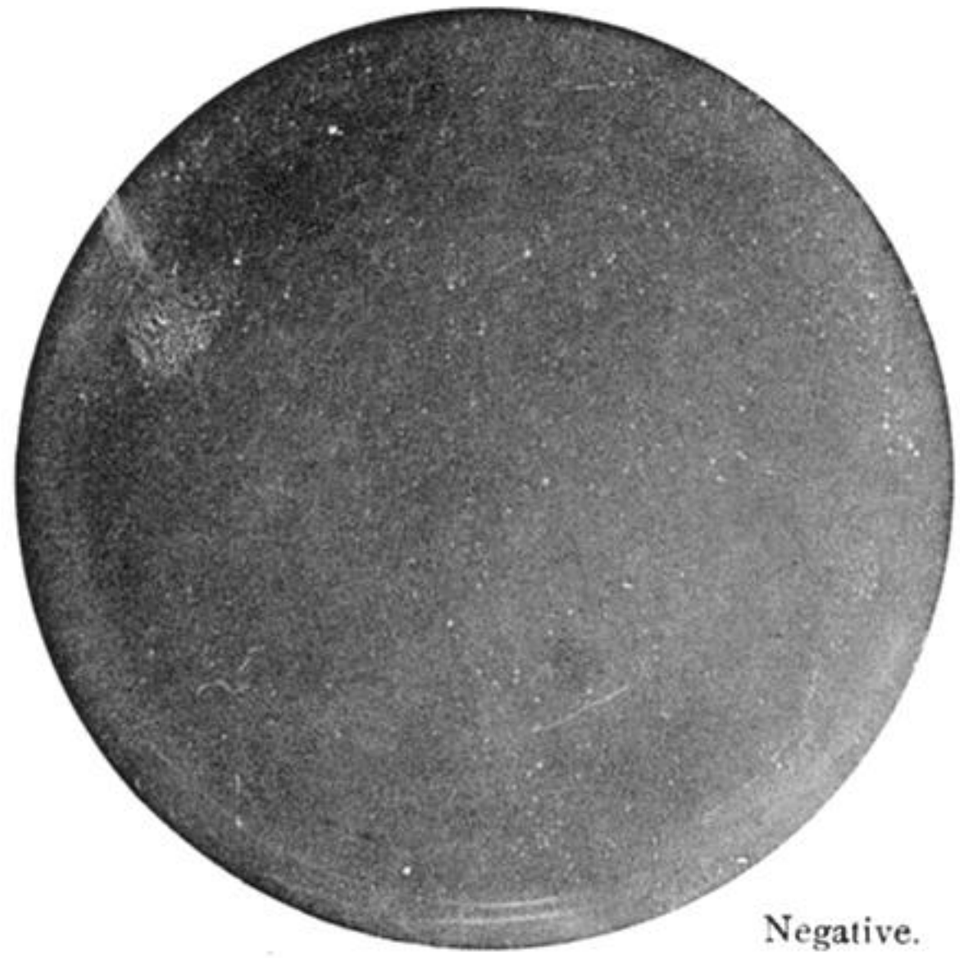
FIG. 10.
Pneumococcus. 70 mins.



Control.

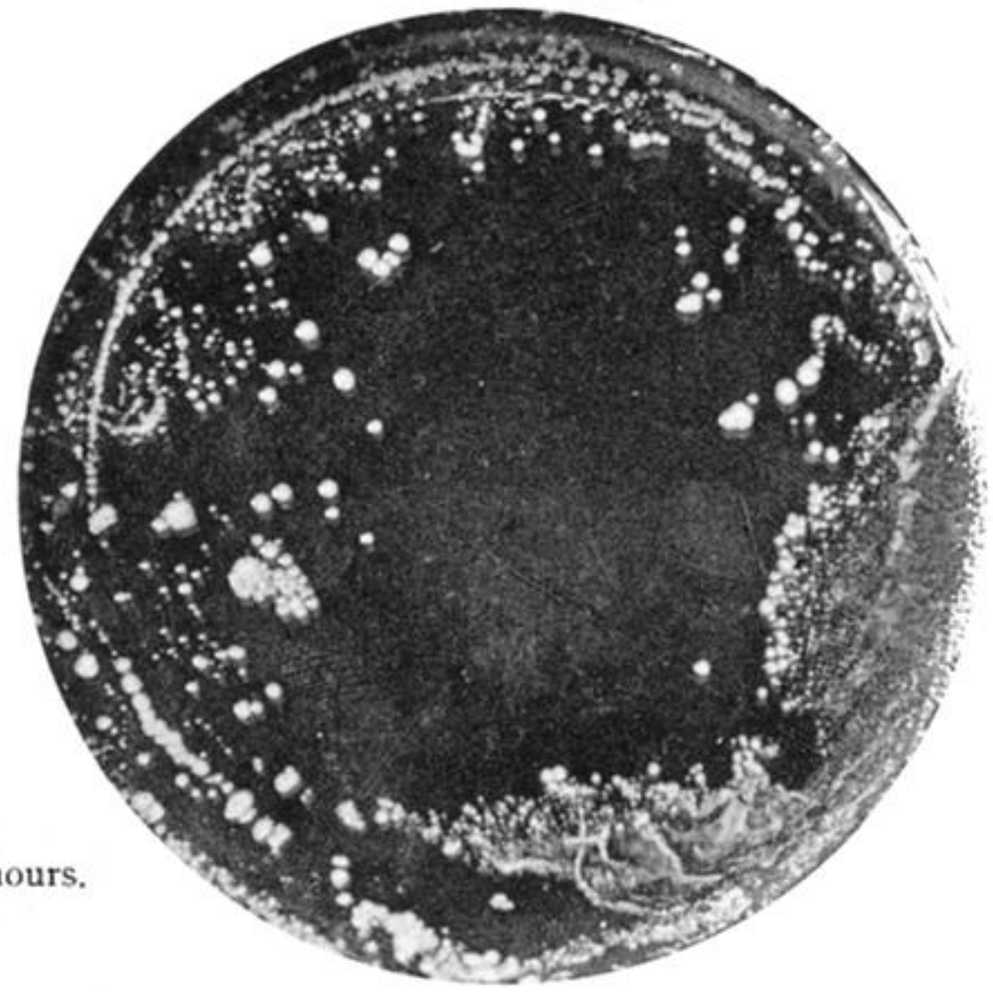


Positive.



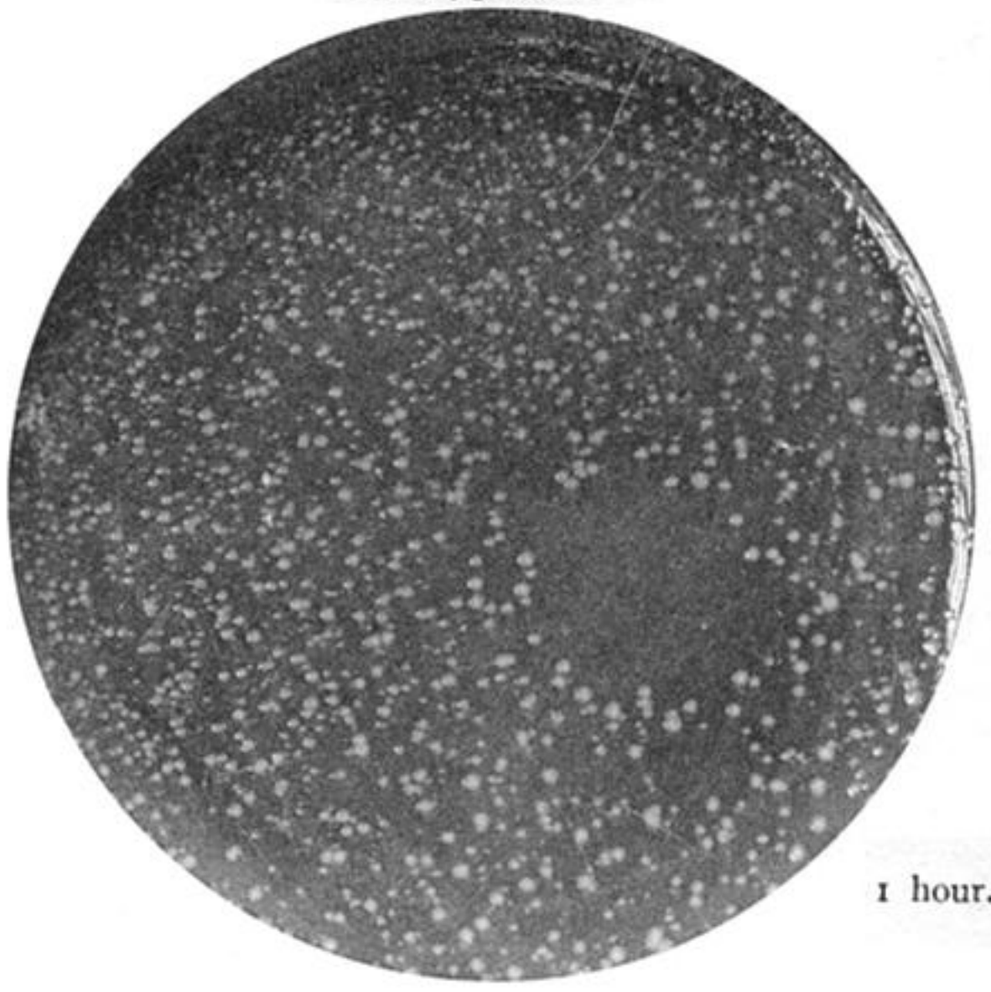
Negative.

FIG. 11.
B. Coli Communis.

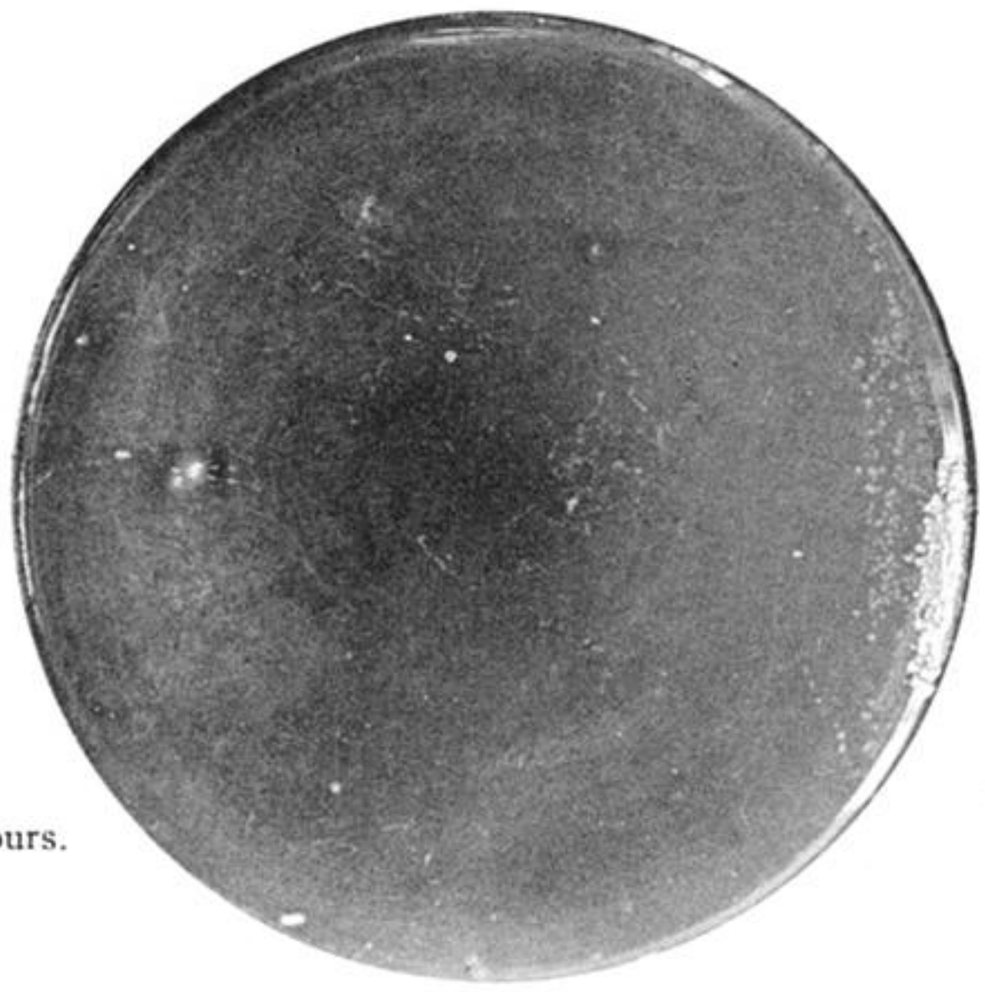


$1\frac{1}{4}$ hours.

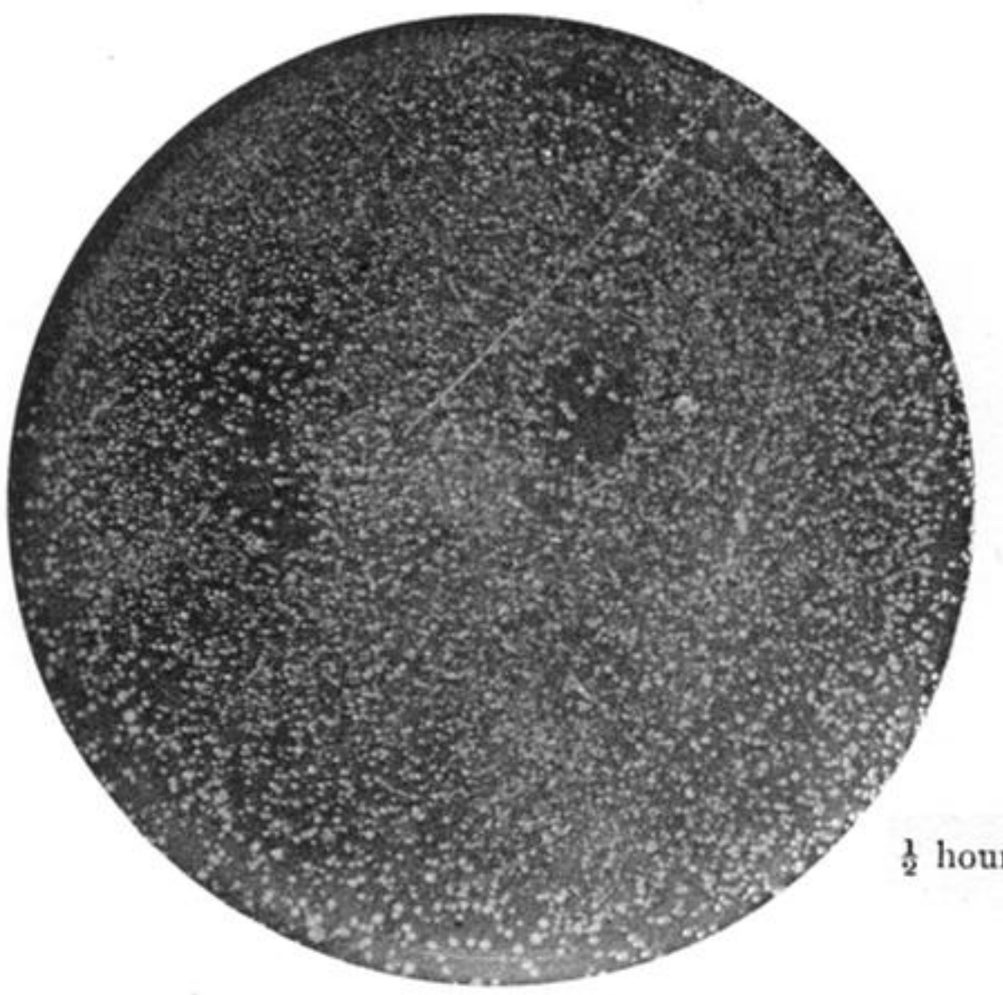
FIG. 12.
B. Typhosus.



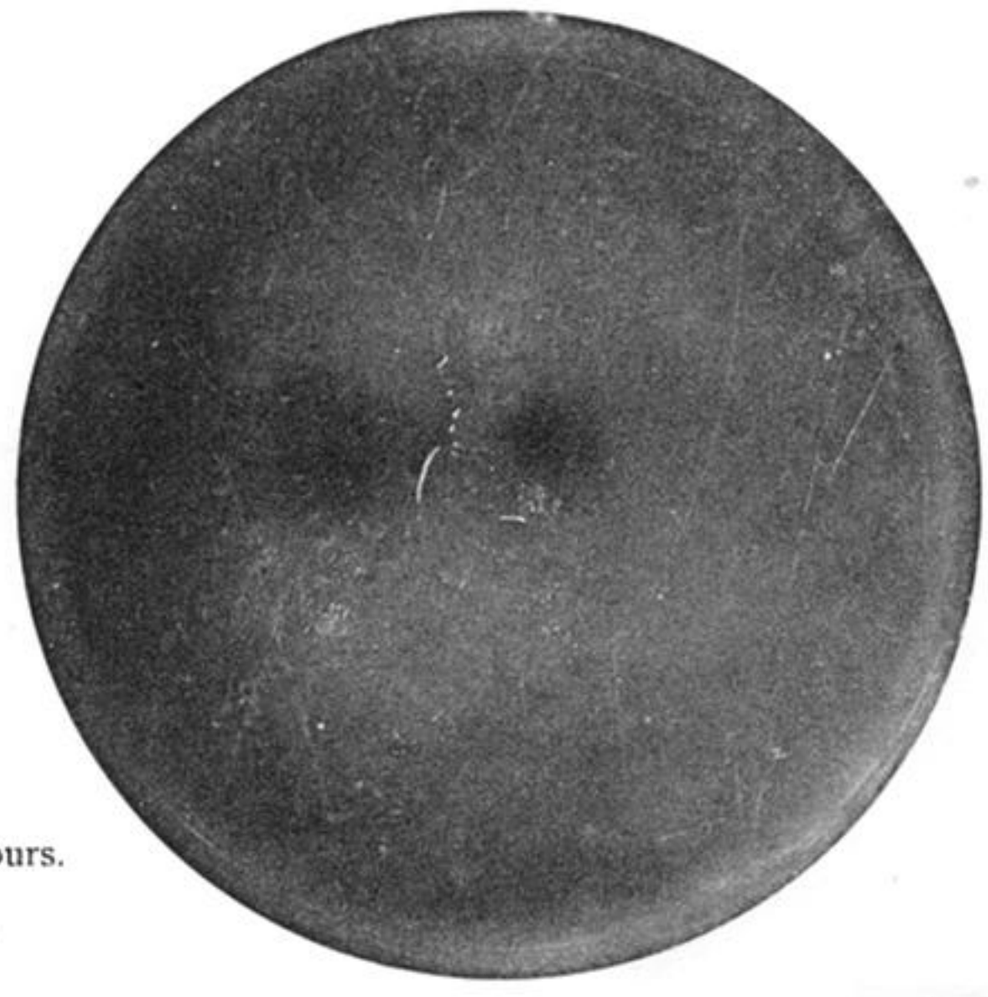
1 hour.



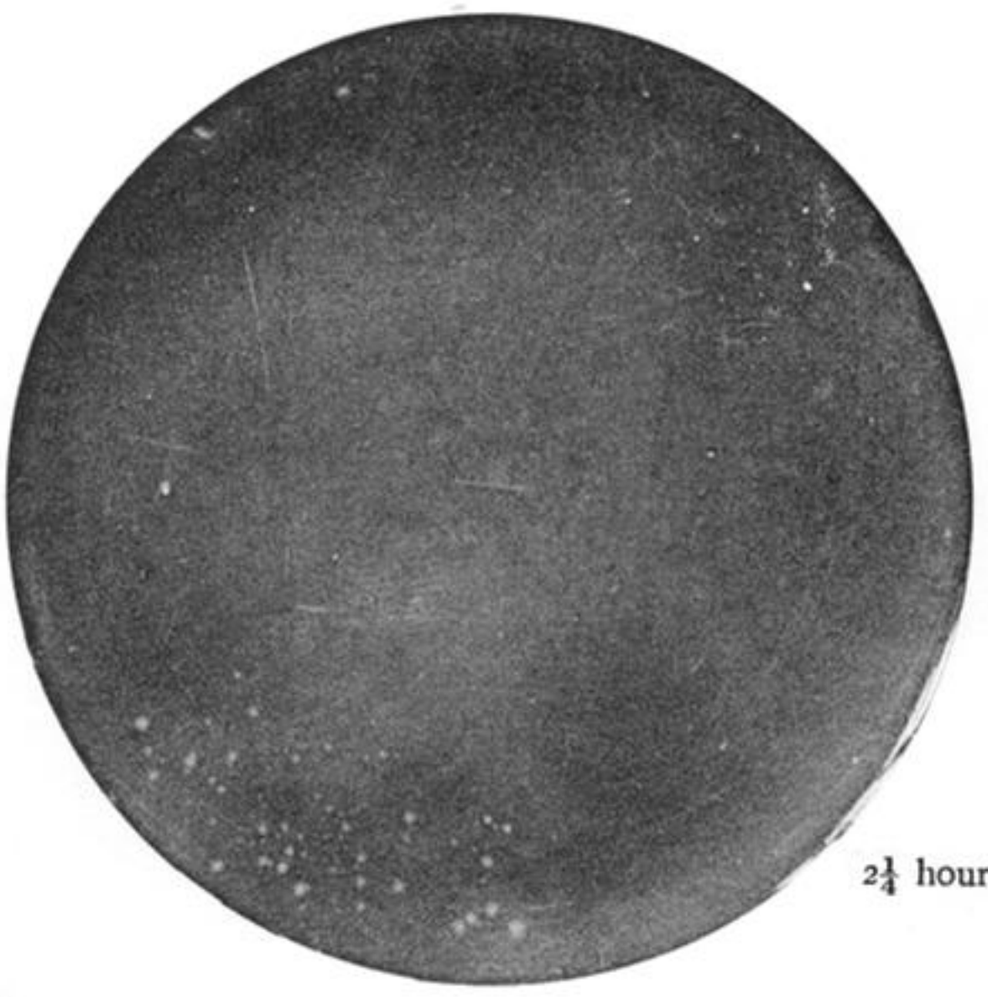
$4\frac{1}{2}$ hours.



$\frac{1}{2}$ hour.

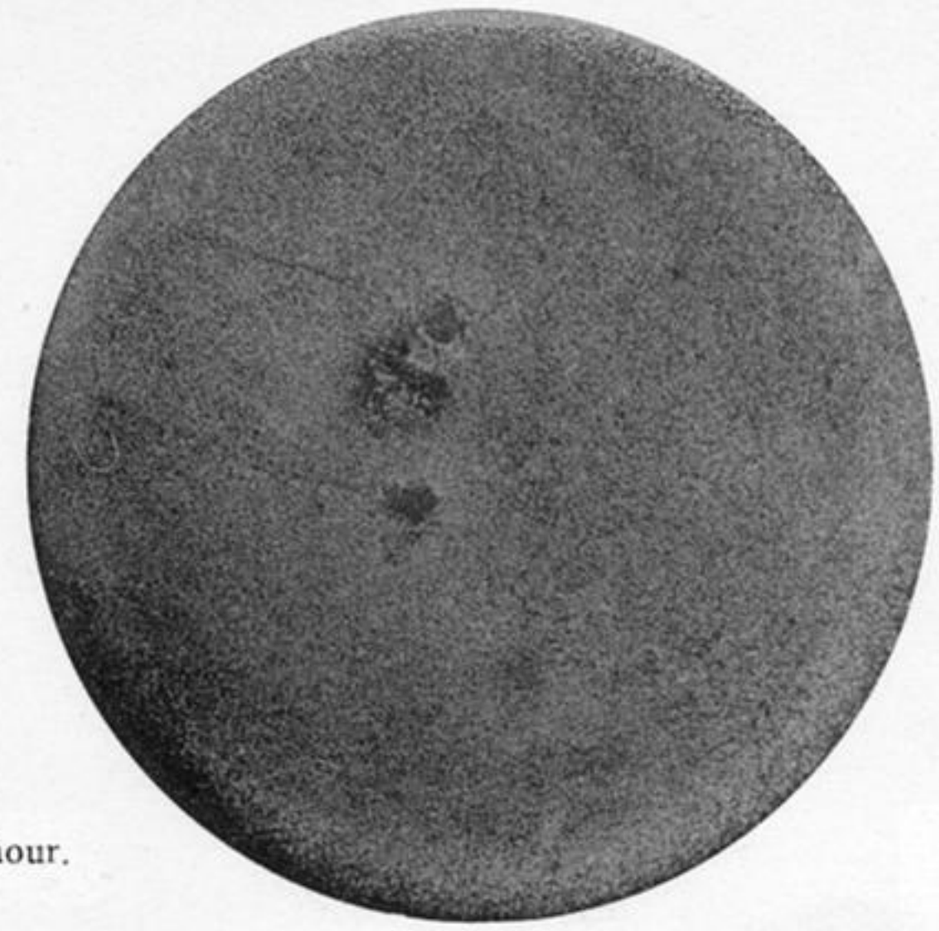


18 hours.



$2\frac{1}{4}$ hours.

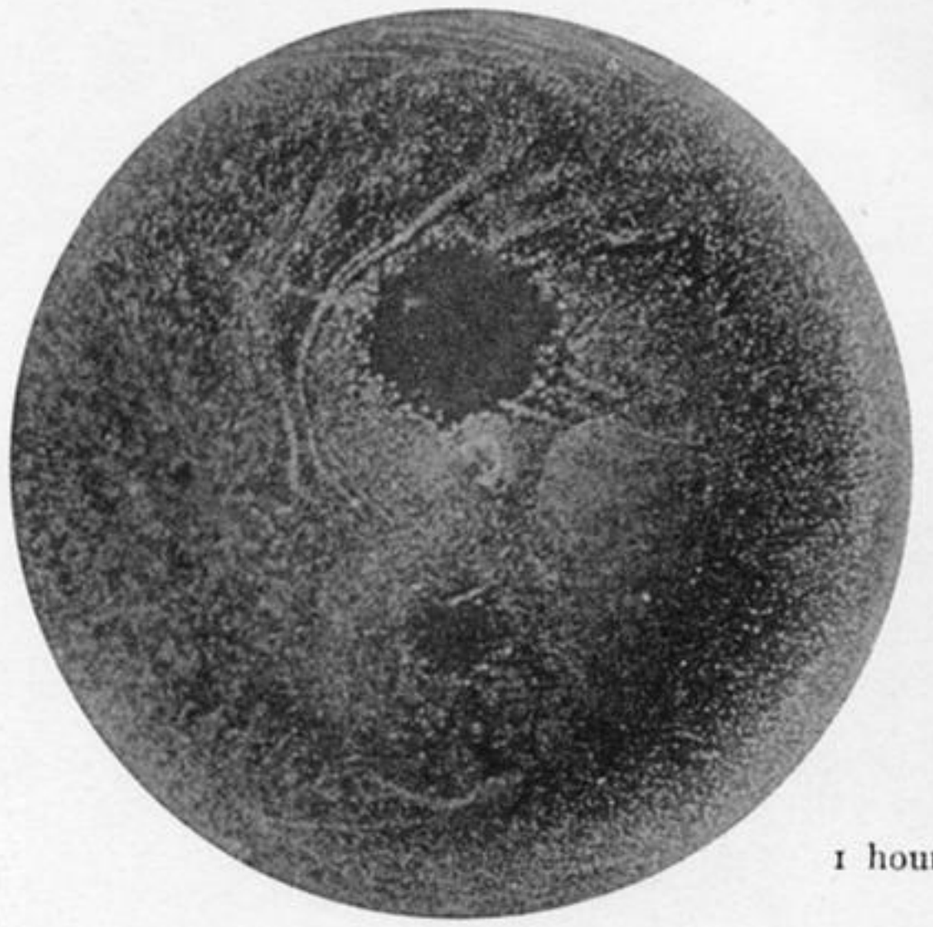
Exposed as in Plate 8.



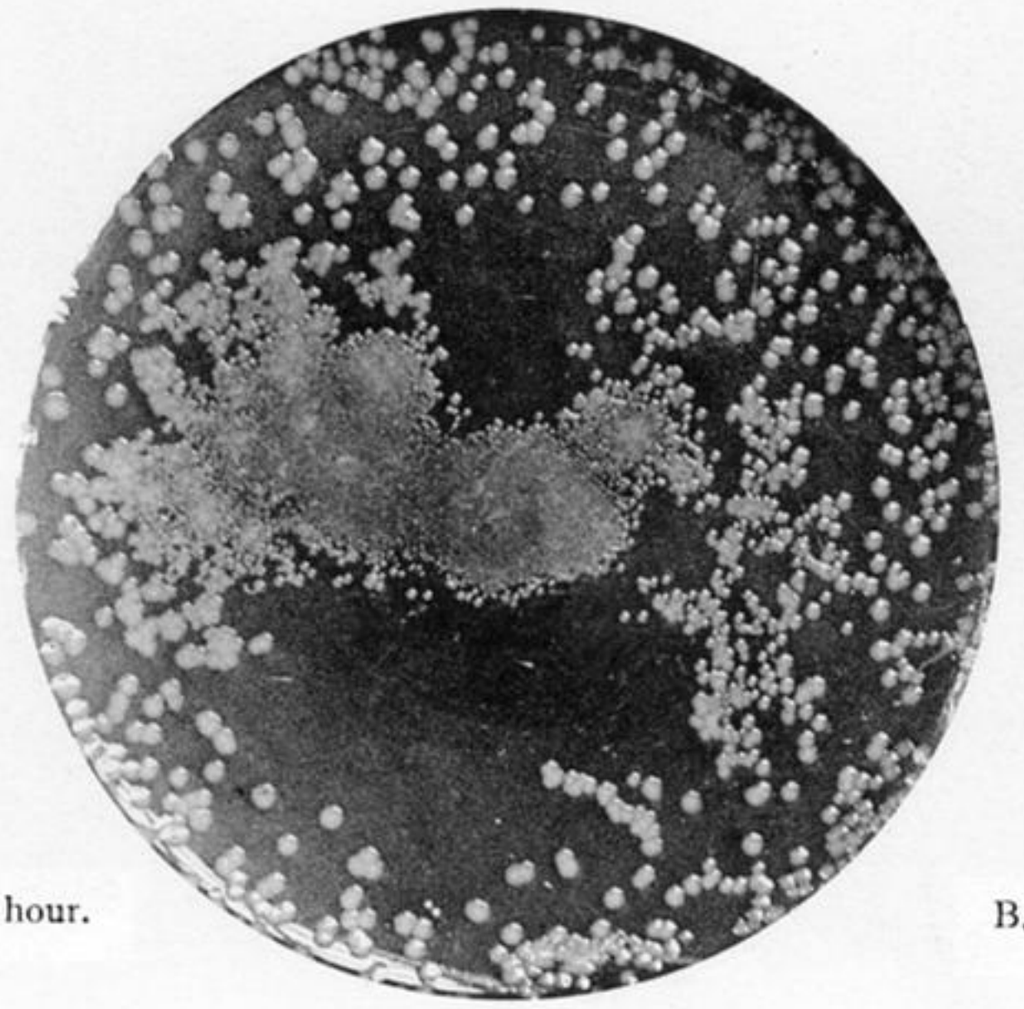
½ hour.

FIG. 13.

B. Coli Communis.



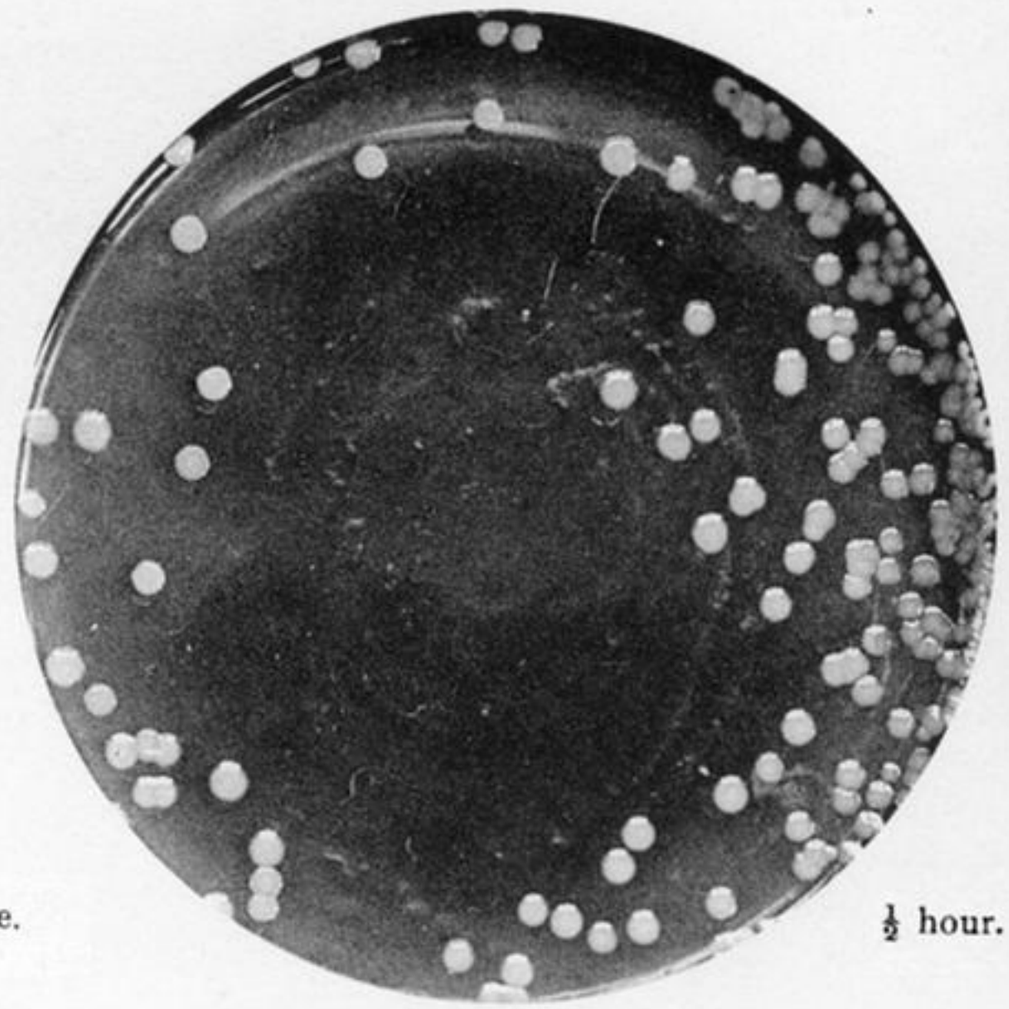
1 hour.



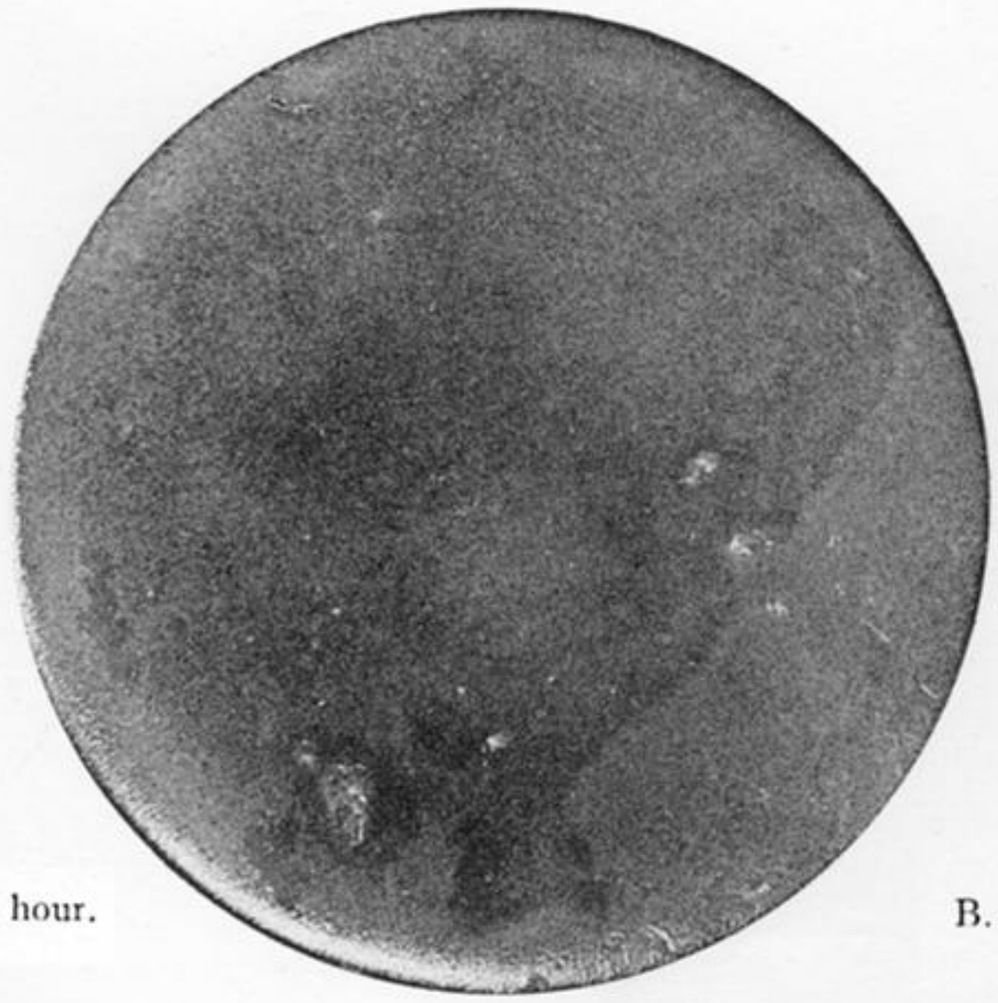
¼ hour.

FIG. 14.

B. Asiat. Choleræ.



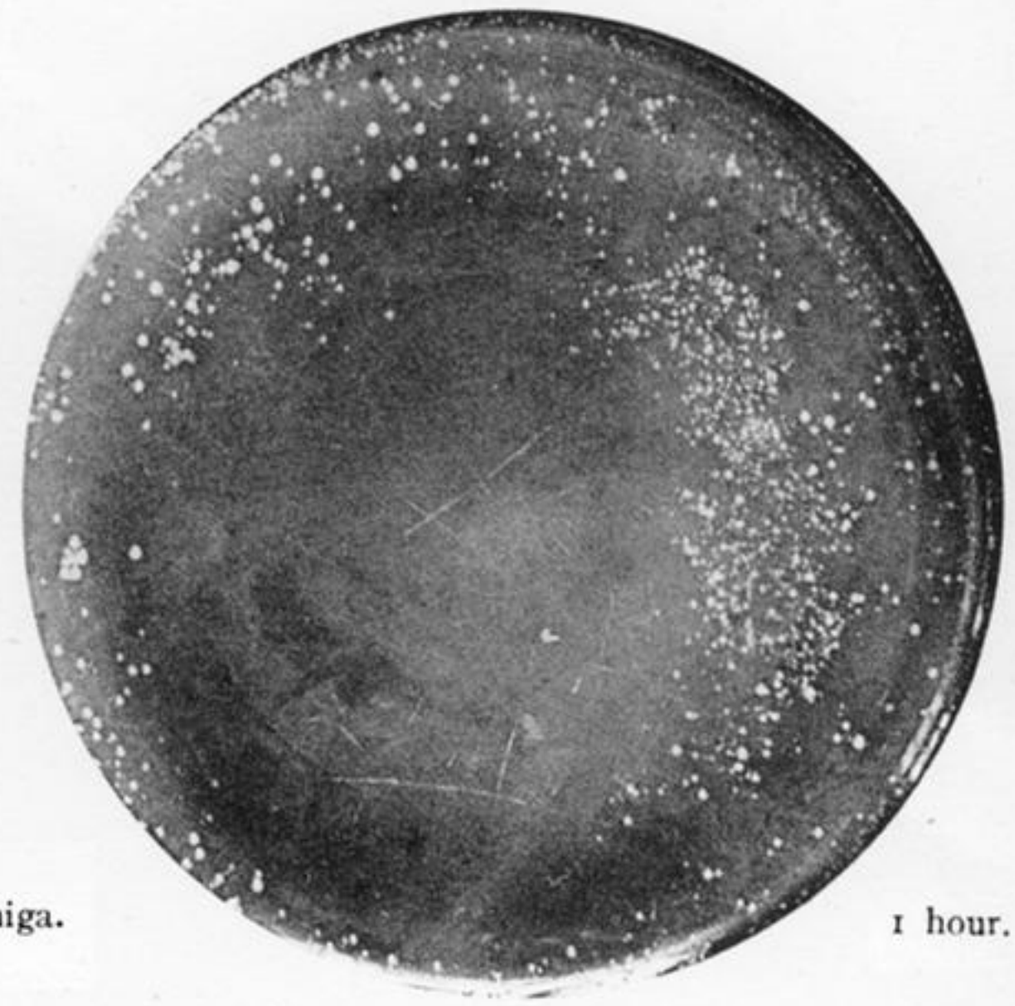
½ hour.



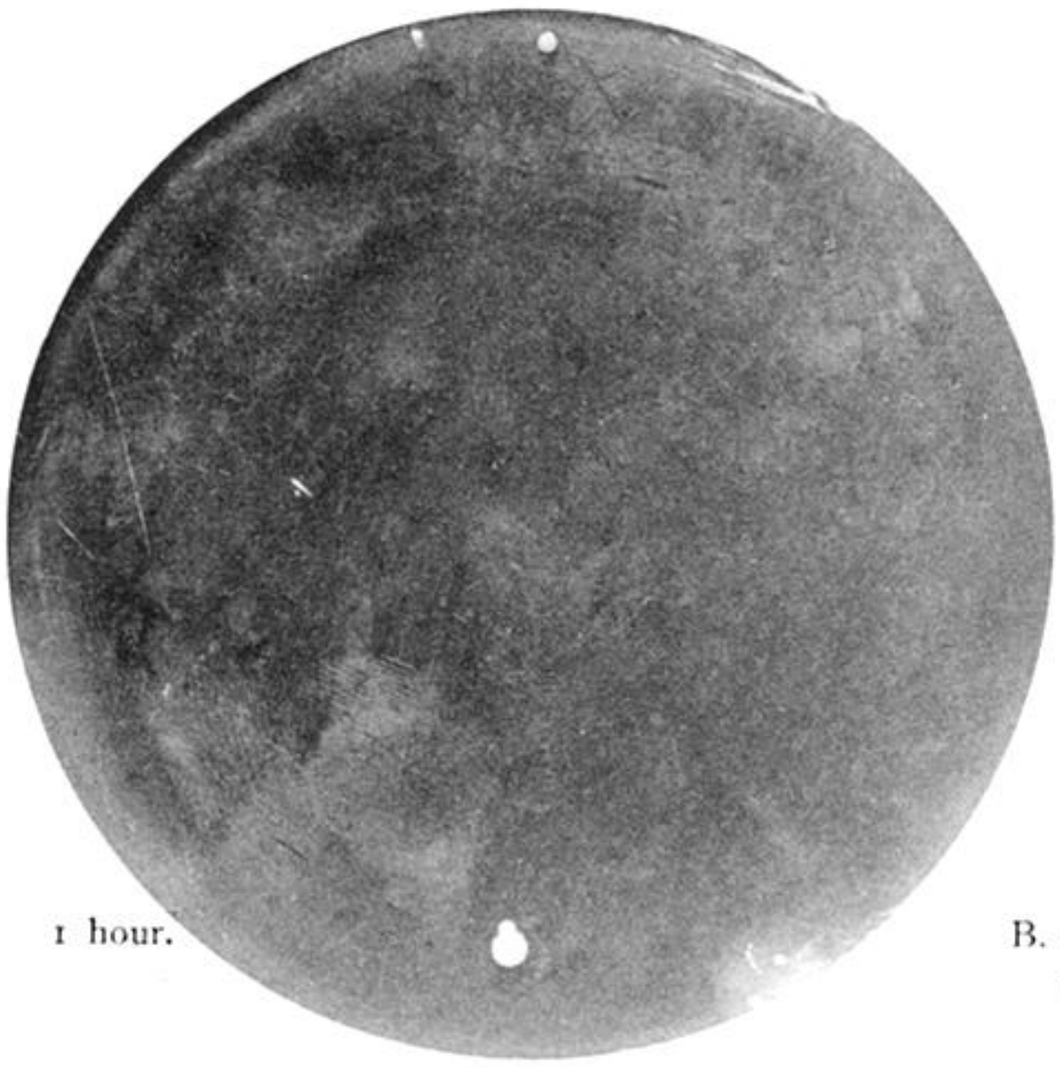
½ hour.

FIG. 15.

B. Dysenterica, Shiga.



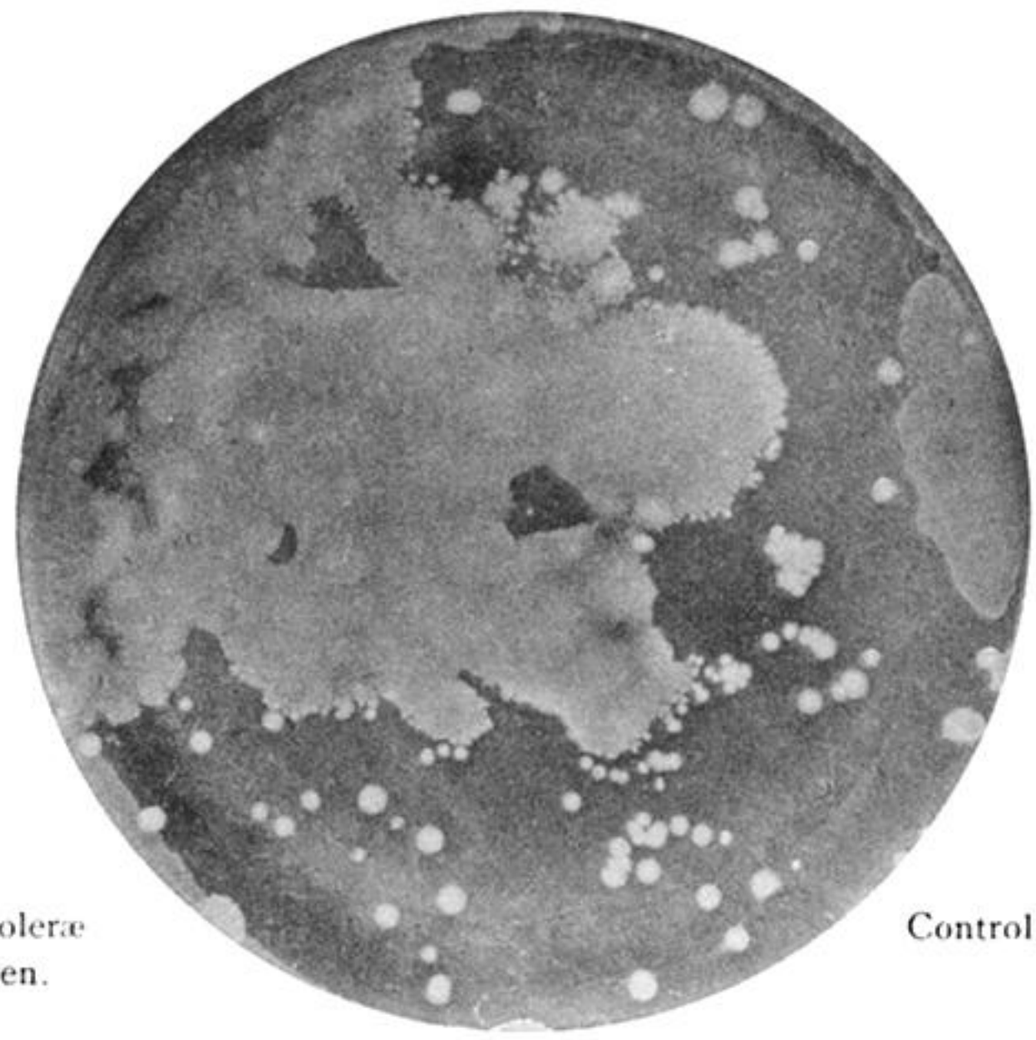
1 hour.



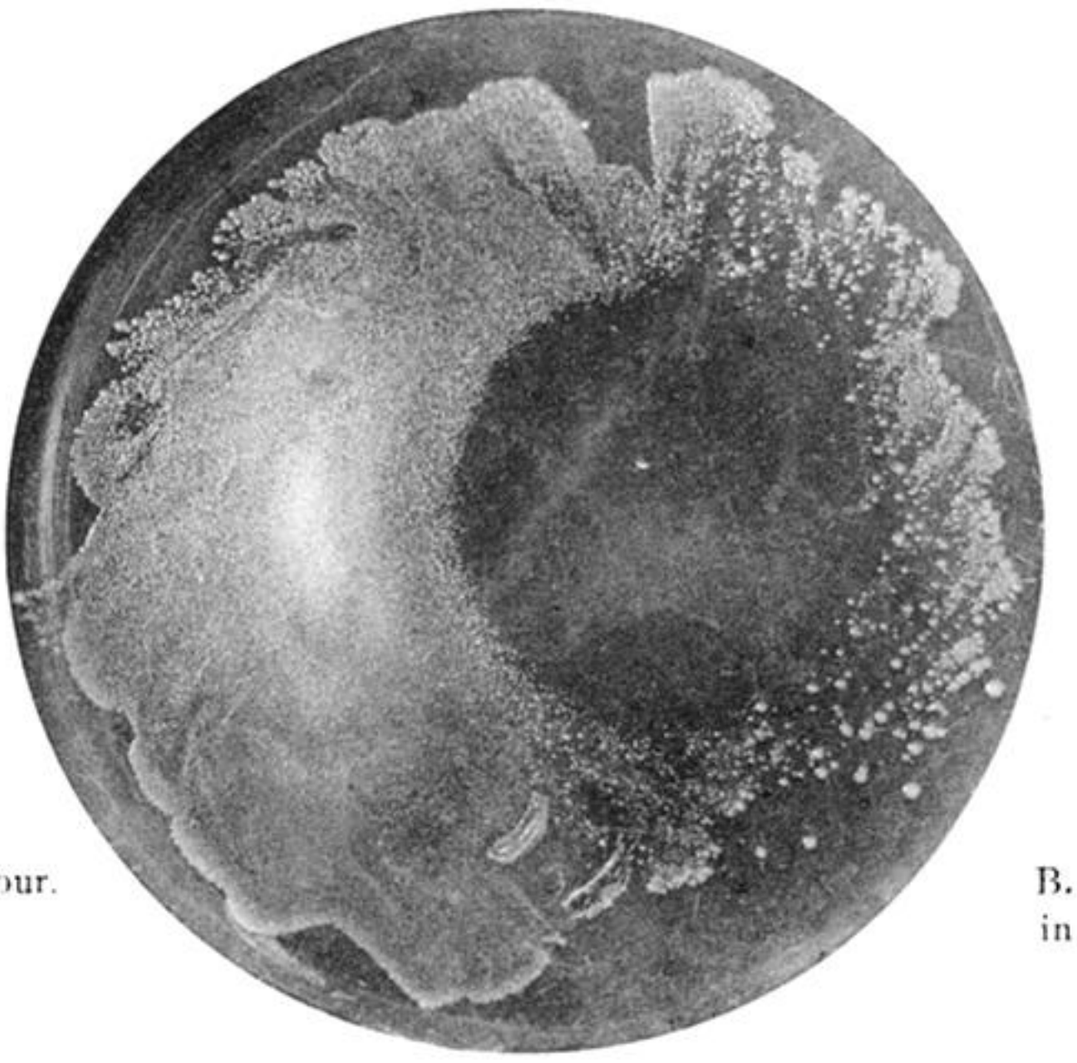
1 hour.

FIG. 16.

B. Asiat. Choleræ in hydrogen.



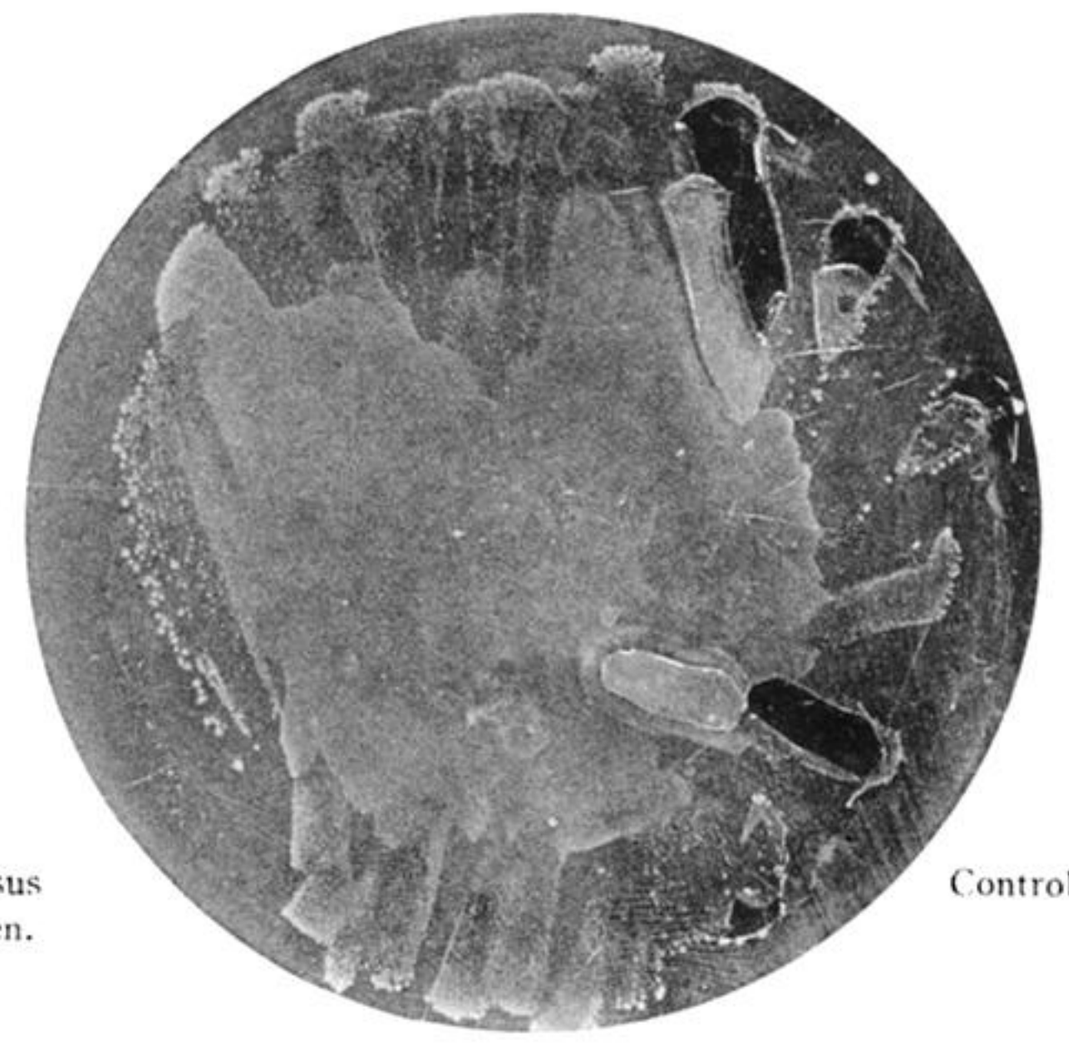
Control.



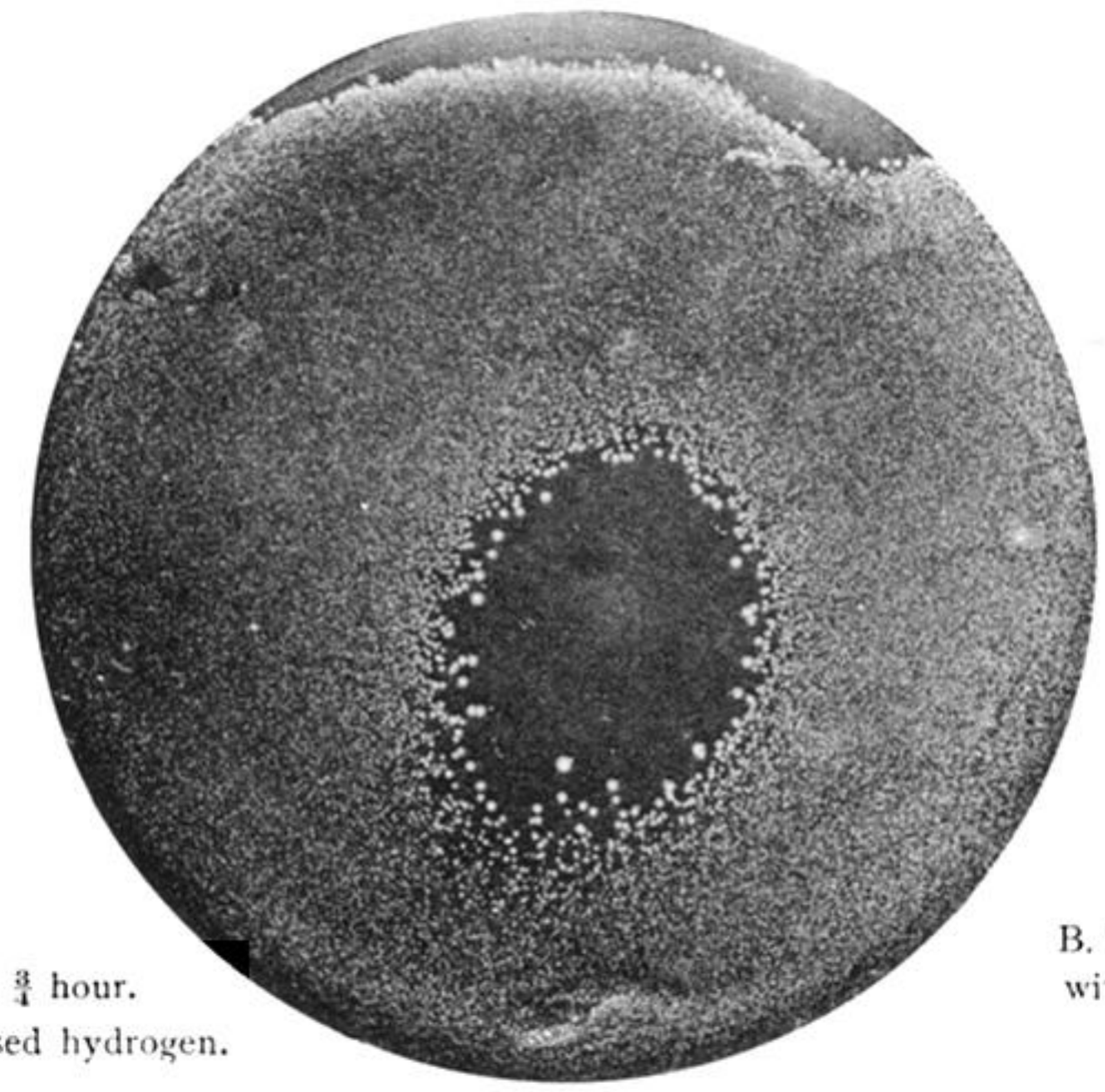
1 hour.

FIG. 17.

B. Typhosus in hydrogen.



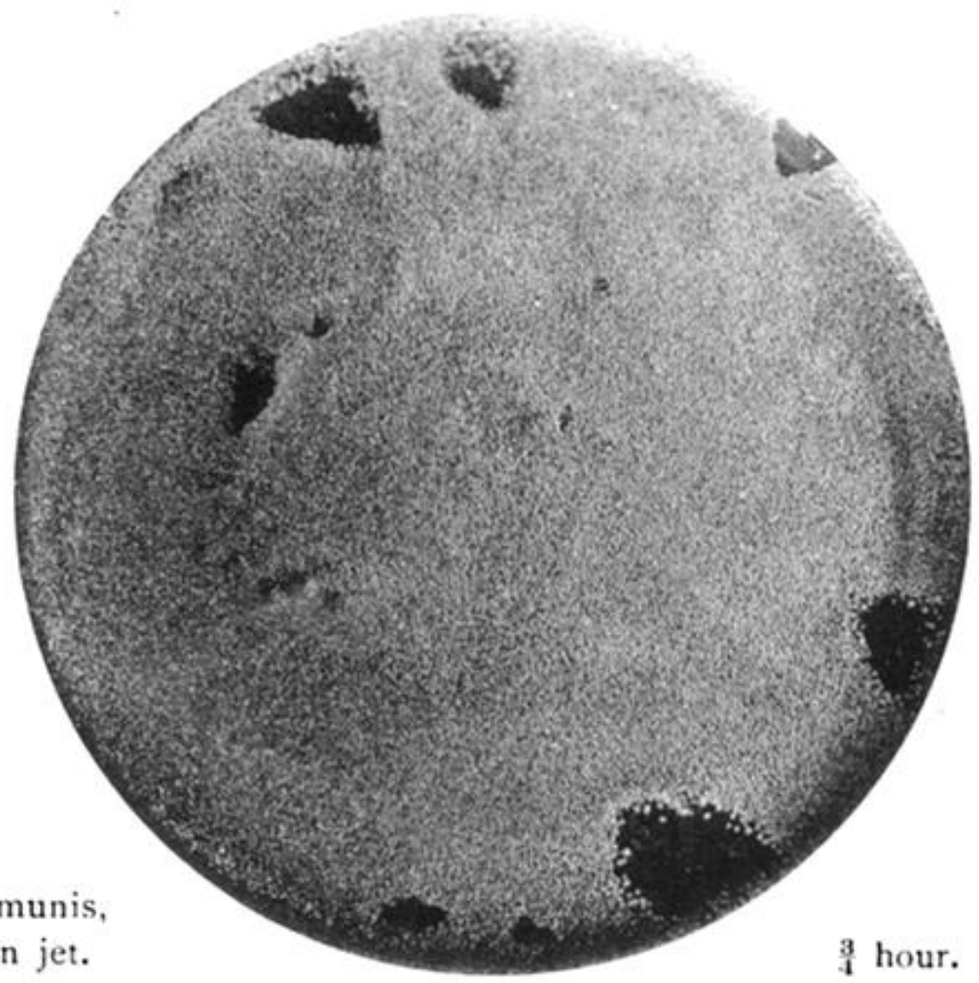
Control.



¾ hour.
Ionised hydrogen.

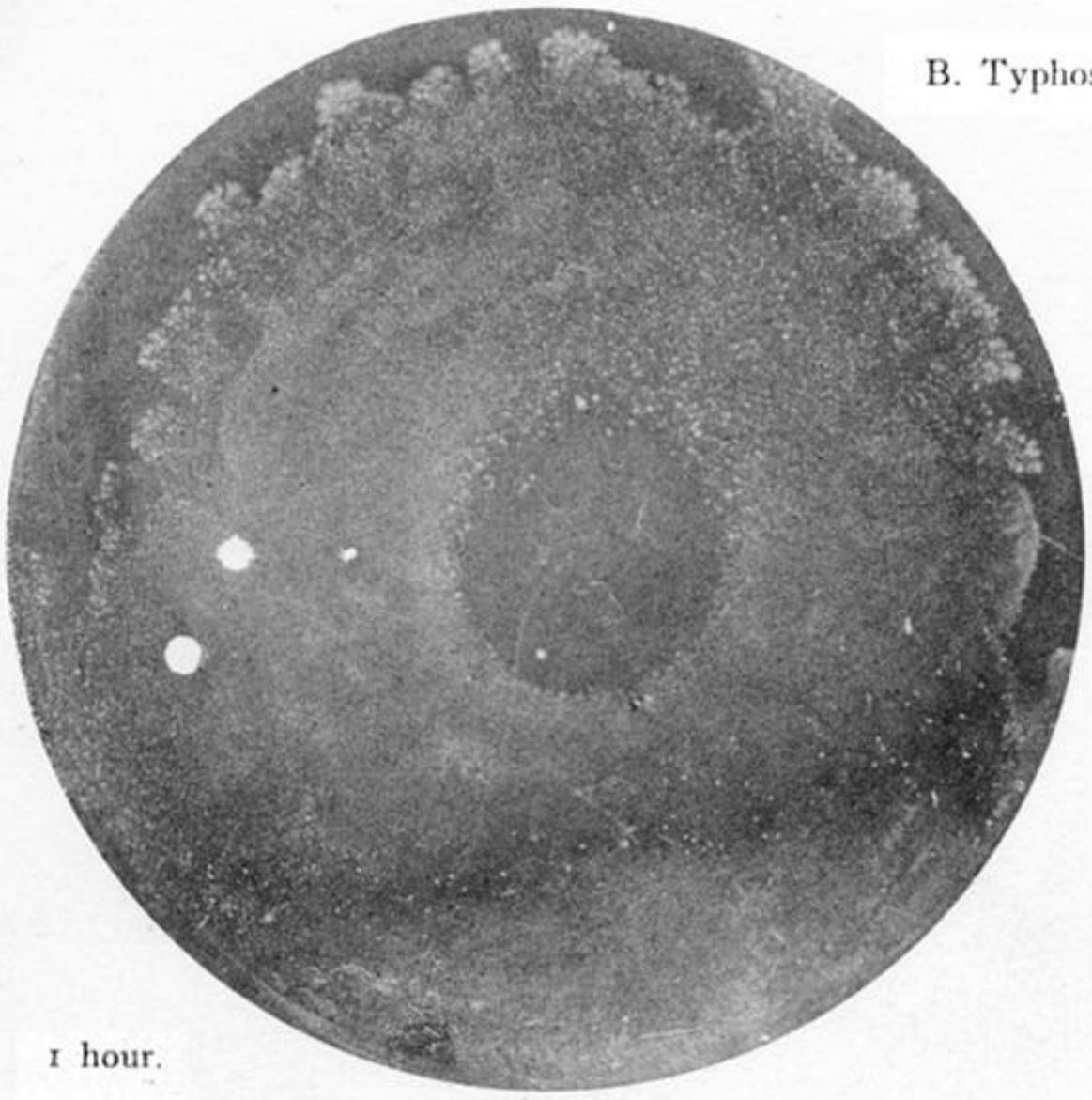
FIG. 18.

B. Coli Communis, with hydrogen jet.



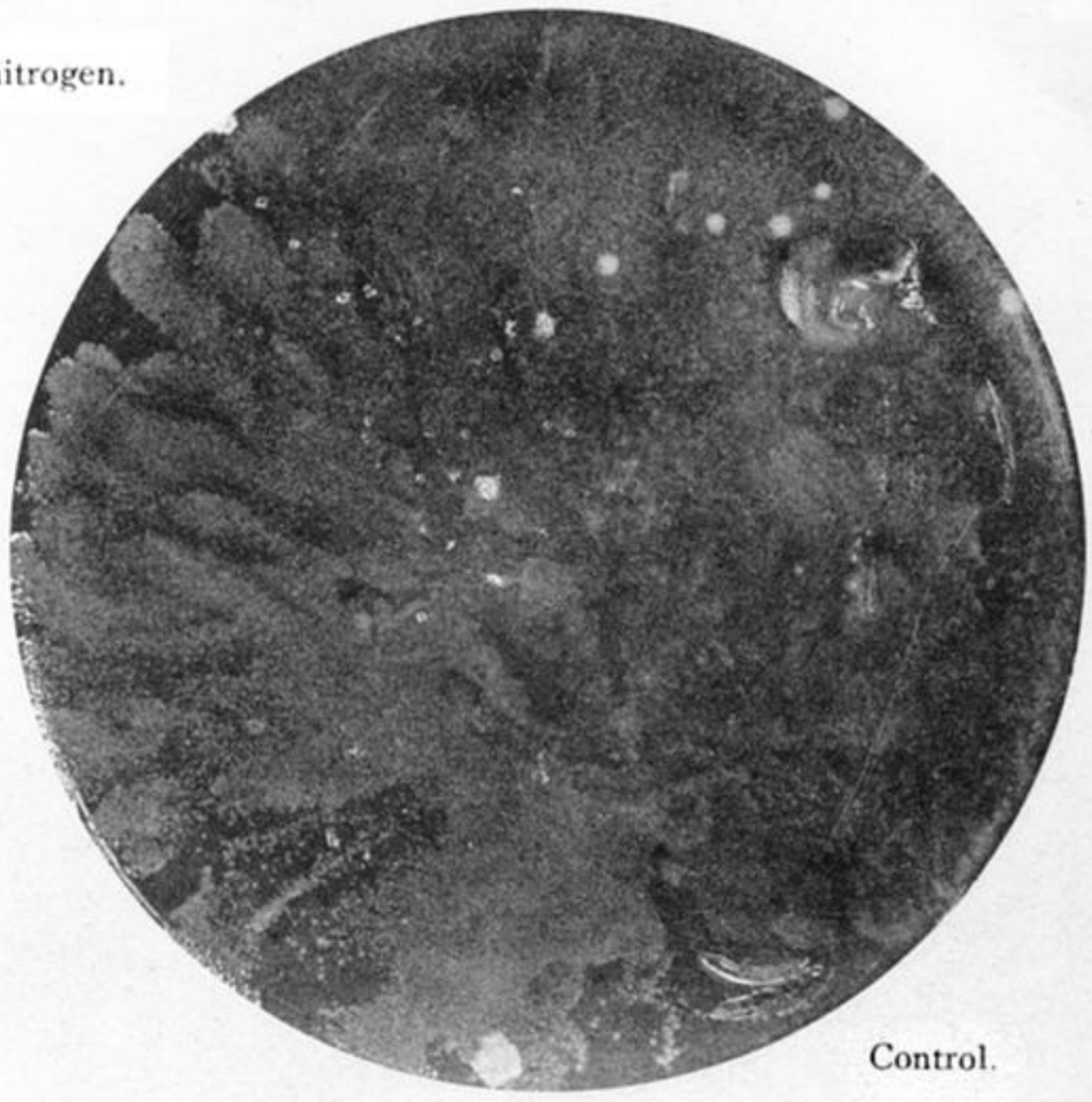
¾ hour.
Hydrogen alone, no effect.

B. Typhosus in nitrogen.



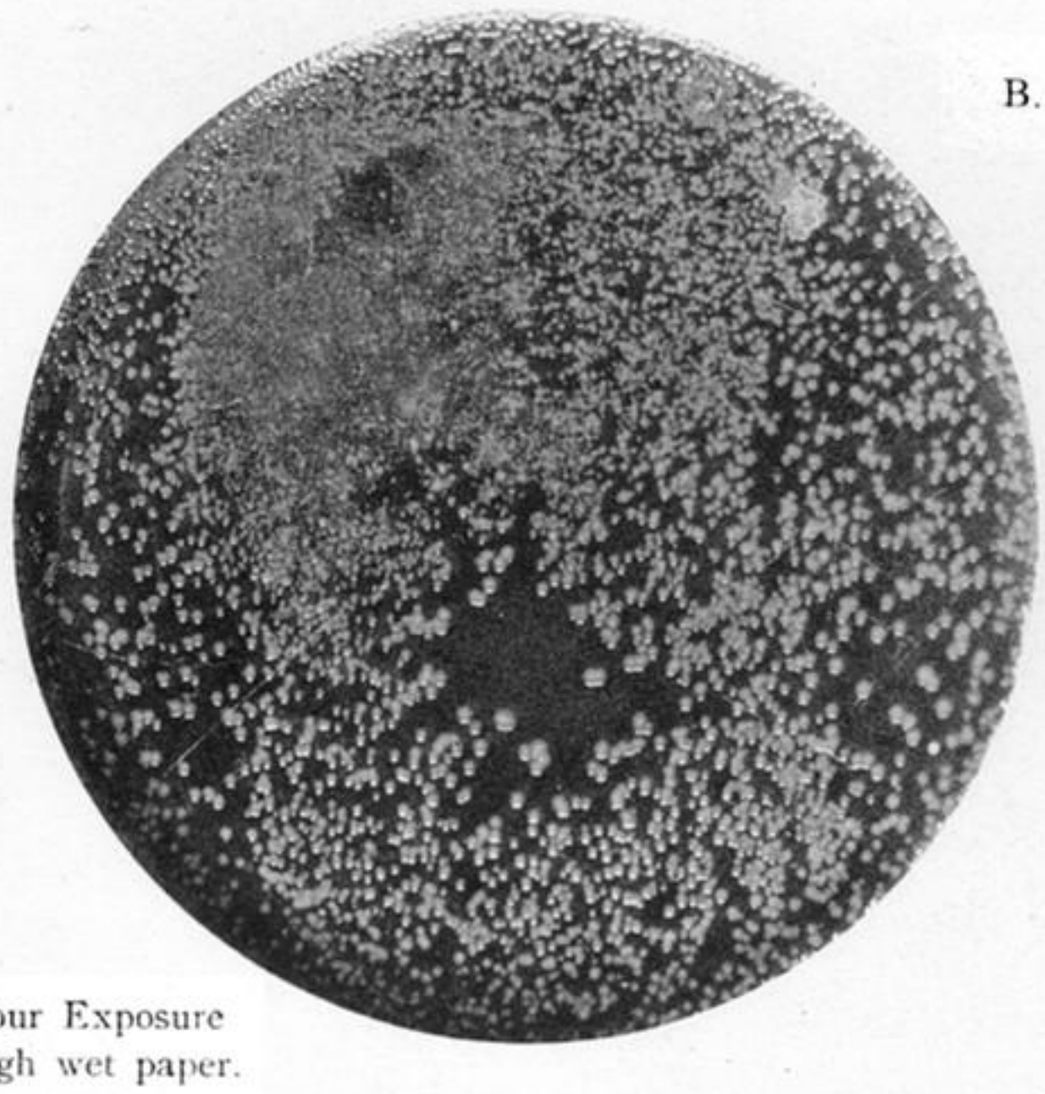
1 hour.

FIG. 20.



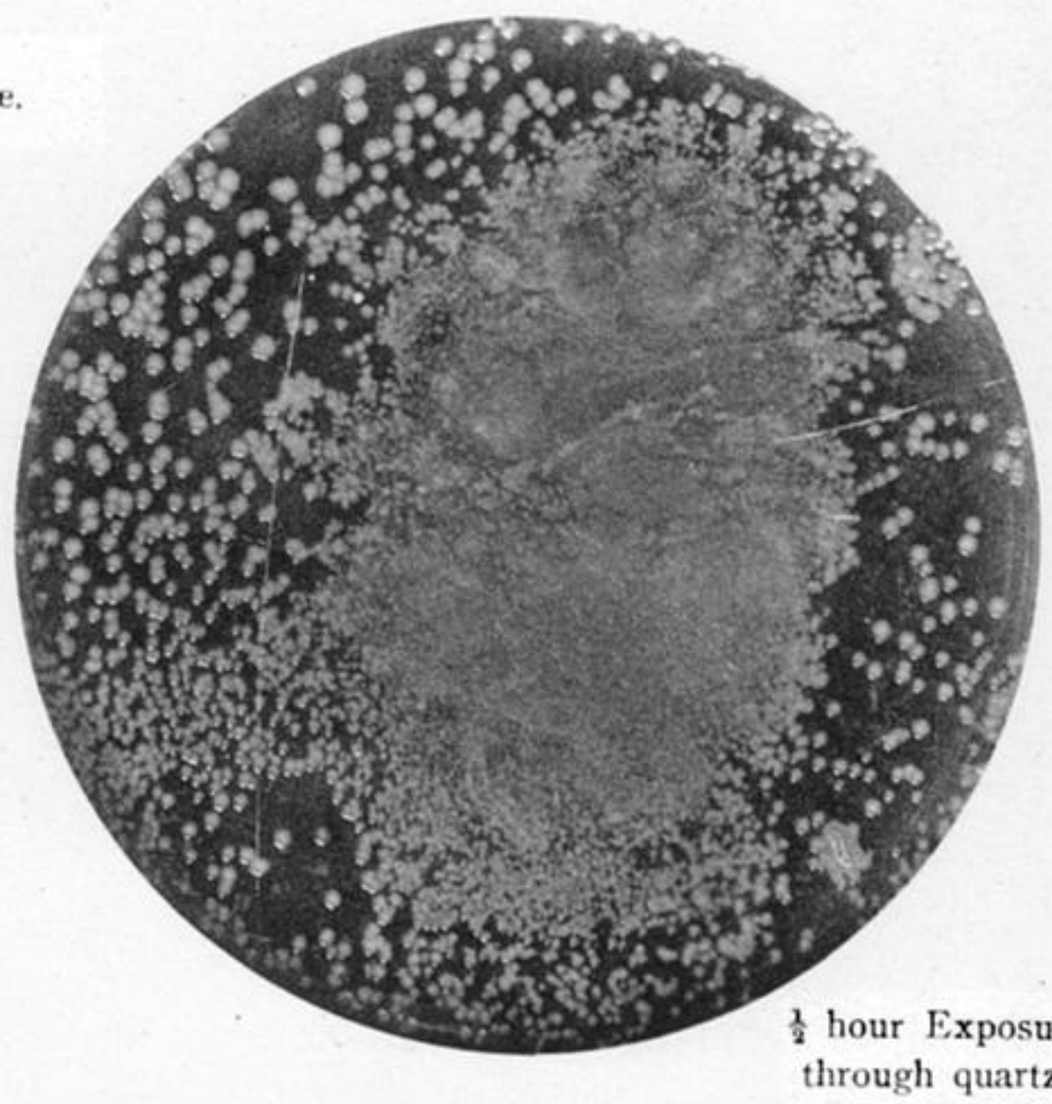
Control.

B. Asiat. Choleræ.



¼ hour Exposure through wet paper.

FIG. 21.



½ hour Exposure through quartz.

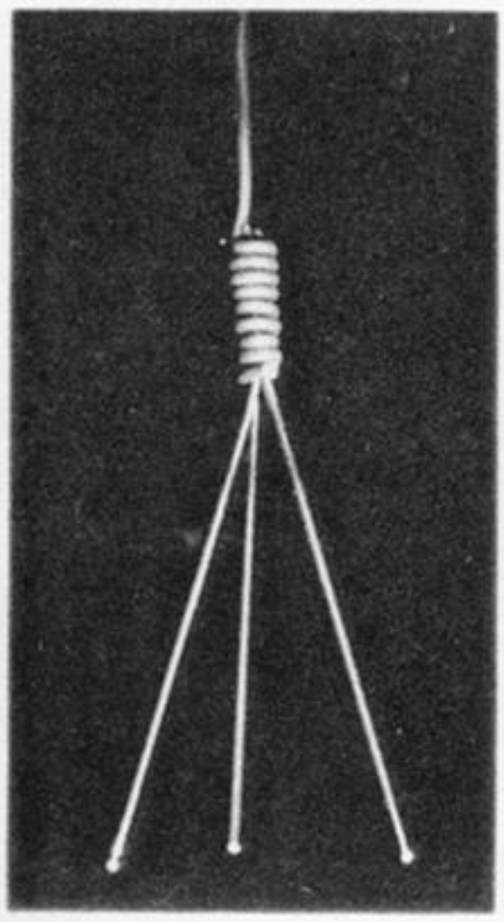


FIG. 3.

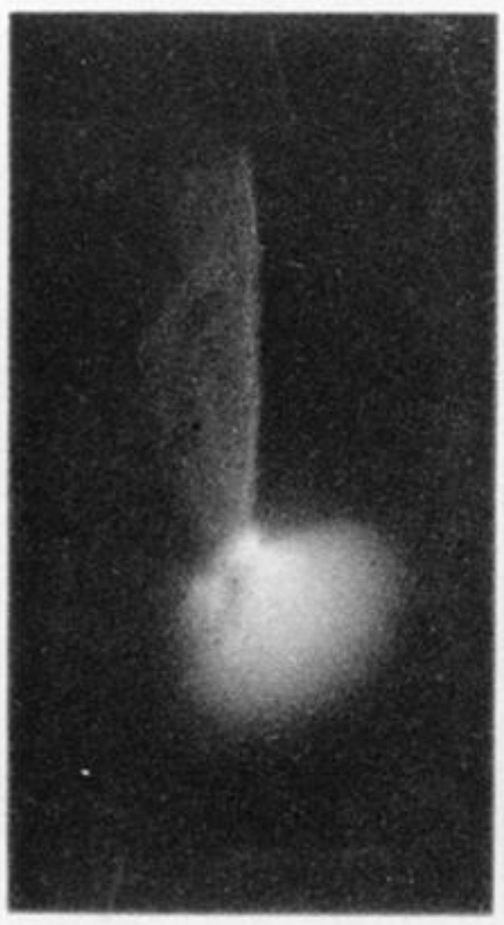


FIG. 4.

FIG. 5.

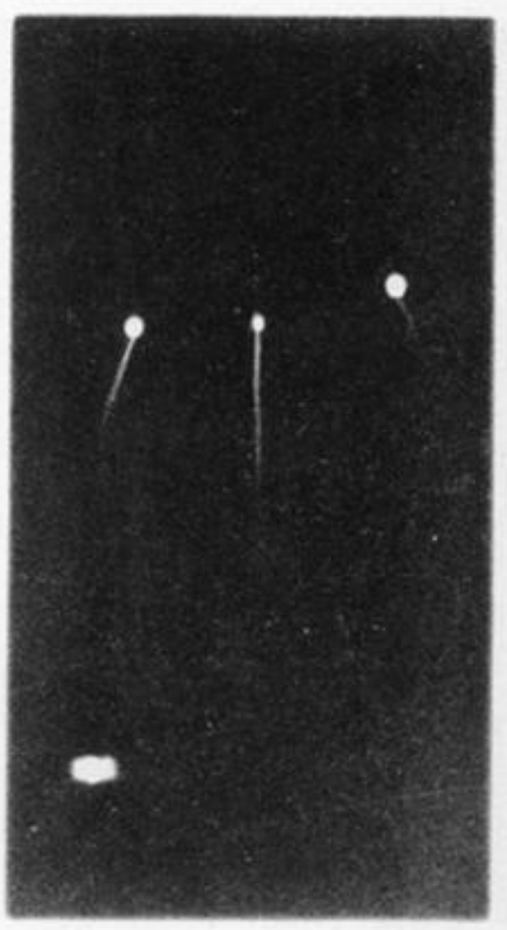


FIG. 6.