On the Toxic Action of Dilute Pure Sodium Chloride Solutions on the Meningococcus.

By Cresswell Shearer, M.D., D.Sc., F.R.S., Military Hospital, Devonport.

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[Plate 15.]

In experimenting during the past season with a large number of freshly isolated strains of the meningococcus, it was noticed that almost all of these were killed, when placed for a short time in dilute pure sodium chloride solutions. This action of sodium chloride is most toxic to the meningococcus when the concentration of the salt is not below 0·85 per cent., and not much above 0·9 per cent. NaCl.

It was found that freshly isolated meningococci were more vulnerable to this action of NaCl, than old laboratory cultures. While old laboratory cultures could sometimes resist the action of a pure 0·85 per cent. NaCl for three or four hours, freshly isolated strains seldom resisted its action for more than 20 minutes.

It seemed remarkable that NaCl should be toxic for the meningococcus in just that concentration which it is accustomed to in the fluids of the body. It is strange that this germ should be so sensitive to the toxic action of sodium chloride, while at the same time it is able to resist for many hours the action of distilled water.

To demonstrate the toxic action of a physiological saline solution on the meningococcus, it is highly important to observe certain conditions in performing the experiment. It is essential, in the first place, that the NaCl used should be free from any impurity. In the second place, the meningococci must be added to such a quantity of the saline solution that any traces of salts brought over with them from the culture medium will have no appreciable effect in antagonising the action of the NaCl. Thirdly, it is very important that the saline is not unduly diluted below its proper toxic strength by the addition of too large a quantity of the emulsion containing the germs. In the fourth place, no agglutination of the germs into masses or clumps in the saline must take place. The saline is unable to act on the germs in the interior of these clumps, so that all are killed. Care must be taken to shake up the germs in the saline thoroughly, and avoid all clumping as much as possible.

I have found from a large number of experiments that one or two drops of an emulsion (of 5000 million meningococci to the cubic centimetre in

distilled water) is the proper quantity of emulsion to be added to 2 c.c. of 0·85 per cent. saline to effectively demonstrate the toxic action of pure NaCl on the meningococcus.

The toxic action of a 0·85 per cent. NaCl solution on the meningococcus can be readily antagonised and rendered harmless by the addition of a very small trace of some bivalent salt, such as $CaCl_2$, with or without the addition of a small quantity of KCl. This is clearly shown by the following experiment, which has been repeated many times.

A twenty-four-hour culture of a recently isolated strain of meningococcus "Lake" was emulsified in distilled water; a fairly thick emulsion being prepared (about 5000 million cocci to the cubic centimetre). Into four sterile test-tubes, capable of being placed in a centrifuge, the following solutions, with 25 cu. mm. of the meningococcus emulsion, were placed as follows:—

1. 2 c.c. sterile 0·85 per cent. NaCl.
2. 2 c.c. „ 0·85 „ NaCl + 0·004 c.c. M/1 $CaCl_2$.
3. 2 c.c. „ 0·85 „ NaCl + 0·004 c.c. M/1 $CaCl_2$ + 0·01 c.c. M/1 KCl.
4. 2 c.c. „ distilled water.

Each tube was then thoroughly shaken to ensure thorough mixing of the solutions and the emulsion of cocci. They were put in the incubator at 37° C. for an hour and a quarter. They were then taken out and centrifuged hard for 15 minutes, and the deposit in each tube planted out separately, in sterile fashion, on a chocolate plate.* Fig. 1 shows the growth obtained on this plate after incubation for 24 hours at 37° C.

An examination of fig. 1 shows that the meningococcus emulsion placed in the pure 0·85 per cent. NaCl, that is the deposit from the tube 1, has failed to grow, and that this quarter of the plate (marked N.S.), planted out with this deposit, is quite free of colonies. The cocci have been killed by the saline. In the opposite quadrant of the plate to this (marked II on the margin of the plate), which has received a similar quantity of emulsion in 0·85 per cent. NaCl, with the addition of a trace of $CaCl_2$, a thick heavy growth of the meningococcus has taken place, covering the entire surface of this quarter of the plate. The $CaCl_2$ here has completely antagonised the toxic action of the NaCl. In quadrant III, where the saline has received the same quantity of $CaCl_2$ as II, but also a little KCl, growth is still thicker (not very well shown in the photograph). In IV, where the germ was simply allowed to stand for an hour and a quarter in distilled water, growth is good, and the colonies cover closely the entire surface of this quarter of the plate.

* Crowe's "chocolate" or blood-trypagar-glucose medium. See 'Lancet,' November 21, 1915.

This experiment clearly demonstrates the four following points :—

1. The toxic action of a pure 0·85 per cent. NaCl solution on the meningococcus.
2. The antagonistic action of a trace of $CaCl_2$ solution over the toxic action of the NaCl.
3. The accelerating action of KCl, when added to $CaCl_2$, in antagonising the toxic action of NaCl.
4. The relatively harmless action of distilled water on the meningococcus.

So definite is this toxic action of 0·85 per cent. NaCl solution on the meningococcus, that it was found possible to make use of it, very successfully, to destroy all meningococci outside or attached to the surface of leucocytes, by simply washing these several times and allowing them to stand for a few hours in a small bulk of pure saline.*

There is no doubt that in this toxic action of dilute NaCl solution on the meningococcus we are dealing with the poisonous action of the Na cation, so extensively investigated by Loeb,† Wasteneys,‡ Osterhout,§ and others.

It is interesting to find that in the case of the meningococcus, as these investigators have found for other forms of life, this toxic action of NaCl is confined to relatively dilute solutions. In the case of the meningococcus, it is essential that the concentration of the NaCl should not be increased much beyond 0·9 per cent., as after this point its toxic action rapidly decreases. The use of a 1·5 per cent. NaCl solution (one of the standard strengths of this salt employed in opsonic work) is without almost any toxic action on the meningococcus, as shown by the following experiment :—

A fairly thick emulsion of a 24 hours' culture of meningococcus "Pryor" was made (about 5000 million cocci to the cubic centimetre). To 4 c.c. of a pure 1·5 per cent. NaCl solution, 25 cu. mm. of this culture, in distilled water, was added, and thoroughly mixed. To 4 c.c. of 0·85 per cent. NaCl solution a similar quantity of the same emulsion was added and mixed. The two solutions were placed in the incubator at 37° C. for an hour. They were then taken out and centrifuged, and the deposit planted out separately on the surface of a chocolate plate, as shown in fig. 2. This figure shows the resulting growth obtained on this plate after 24 hours' incubation at 37° C.

The 0·85 per cent. NaCl solution (marked N in the plate) has killed the meningococcus, while a good growth has been obtained on that half of

* See paper by Shearer and Crowe, "The *Rôle* of the Phagocyte in Cerebro-spinal Meningitis," 'Roy. Soc. Proc.,' B, vol. 89, p. 422 (1916).

† Loeb, 'Collected Papers,' Part II, University of Chicago, 1906.

‡ Loeb and Wasteneys, 'Journ. Bio. Chem.,' vol. 21 (1915).

§ Osterhout, 'Zeit. f. Physkl. Chem.,' vol. 70 (1910).

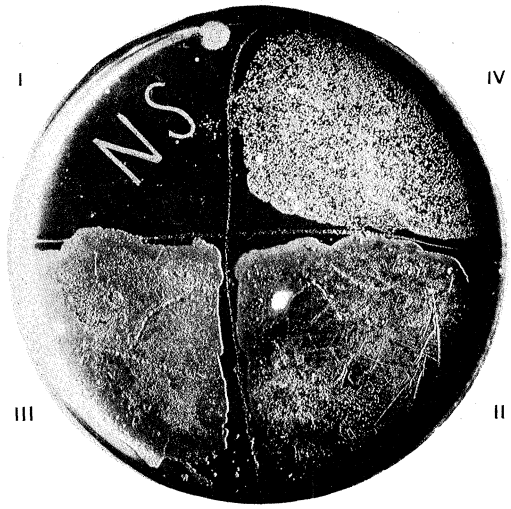


FIG. 1.

1.5% NaCl Sol.

0.85% NaCl Sol.

FIG. 2.

FIG. 3.

the plate inoculated with a similar quantity of the same emulsion of the meningococcus, which had been exposed to the action of a 1·5 per cent. NaCl solution. Thus, as Loeb has found, when the concentration of the NaCl solution increases the toxic action diminishes.

I should like to draw attention, finally, to an experiment made to determine the length of time the meningococcus may remain alive in pure distilled water, as compared with the time it can remain alive in a 0·85 per cent. NaCl solution.

Into 4 c.c. of distilled water and 4 c.c. of 0·85 per cent. NaCl solution respectively, 25 cu. mm. of a meningococcus emulsion was placed. The two solutions were incubated for 24 hours at 37° C., centrifuged down for an hour, and planted out separately on a chocolate plate. In fig. 3 is shown the resulting growth on this plate after 24 hours' incubation at 37° C. The saline, as usual, has killed the germs, while a considerable number of those that have been exposed to the action of the distilled water for 24 hours have survived, and have given rise to an extensive growth. This experiment clearly demonstrates the power of the meningococcus to resist the hypotonic action of distilled water for many hours.

This experiment has been repeated with a large number of different strains of the meningococcus, and it was found that considerable difference in this power of withstanding the action of distilled water was possessed by each strain. It was the exception, however, to find a strain which did not survive the exposure to the action of distilled water at 37° C. for three hours.

DESCRIPTION OF PLATE 15.

FIG. 1.—Photograph of the growth obtained on a plate of Crowe's chocolate medium after 24 hours' incubation at 37° C., showing the toxic action of a pure 0·85 per cent. NaCl solution on the meningococcus and the antagonistic action of a trace of $CaCl_2$. I. Portion of plate planted out with emulsion of the meningococcus in 0·85 per cent. NaCl solution. No growth whatever has taken place, all organisms being killed. II. Portion of plate planted out with a similar quantity of emulsion in 0·85 per cent. NaCl + a trace of $CaCl_2$; here the $CaCl_2$ has antagonised the toxic action of the NaCl, with a resulting thick growth of the meningococcus. III. Portion of plate planted out with a similar quantity of emulsion in 0·85 per cent. NaCl + $CaCl_2$ + KCl, with a resulting thick heavy growth, more than in II. IV. Portion of plate planted out with a similar quantity of meningococcus emulsion which had been allowed to stand for 1¼ hours in distilled water; good growth.

FIG. 2.—Showing the toxic action of 0·85 NaCl solution on the meningococcus, in distinction to the relatively harmless action of a 1·5 per cent. solution of the same salt. N. Normal saline solution.

FIG. 3.—Showing the action of distilled water in failing to kill the meningococcus after 24 hours. D. Distilled water portion. N.S. Saline portion of the plate; all germs killed.

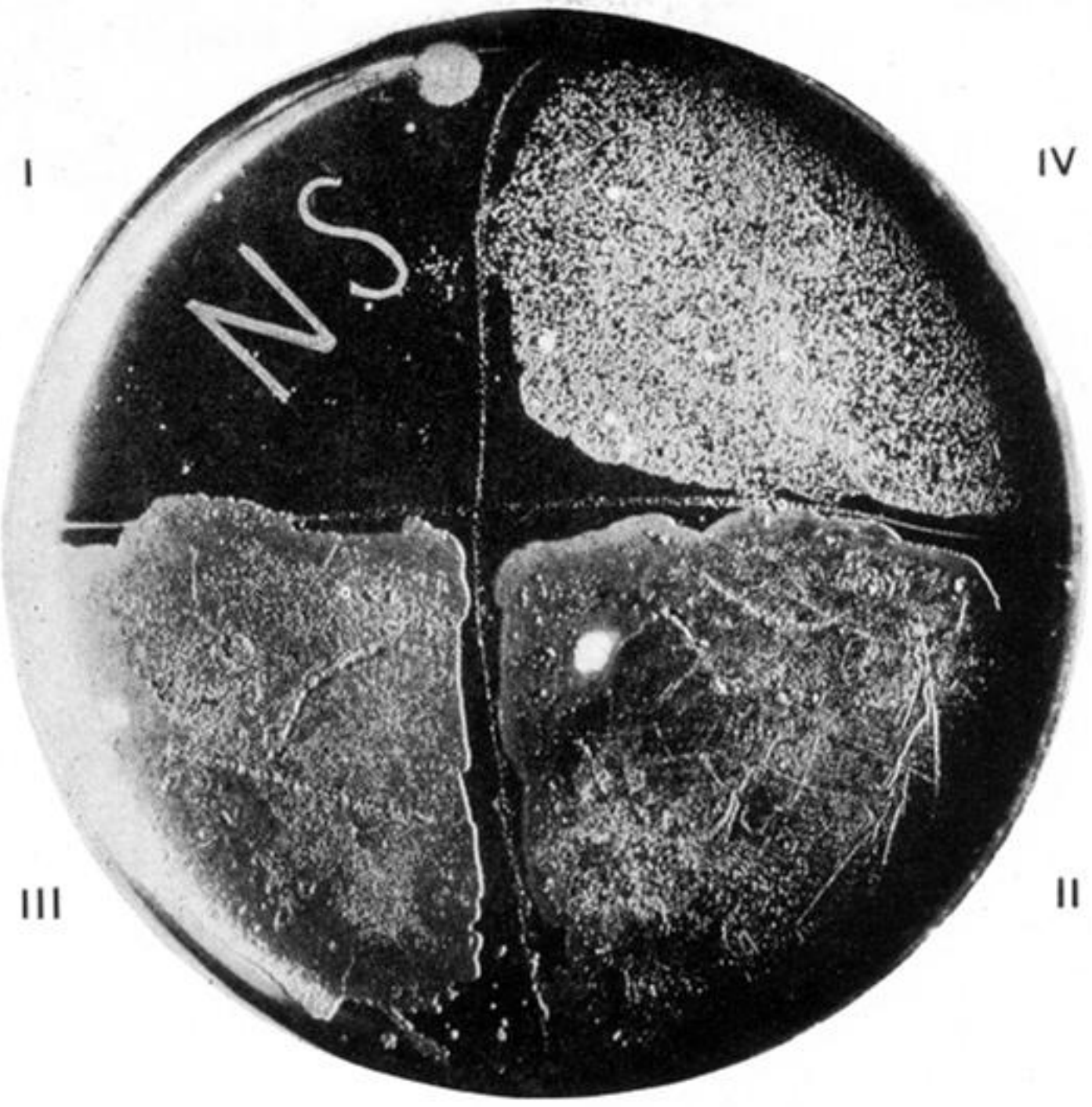
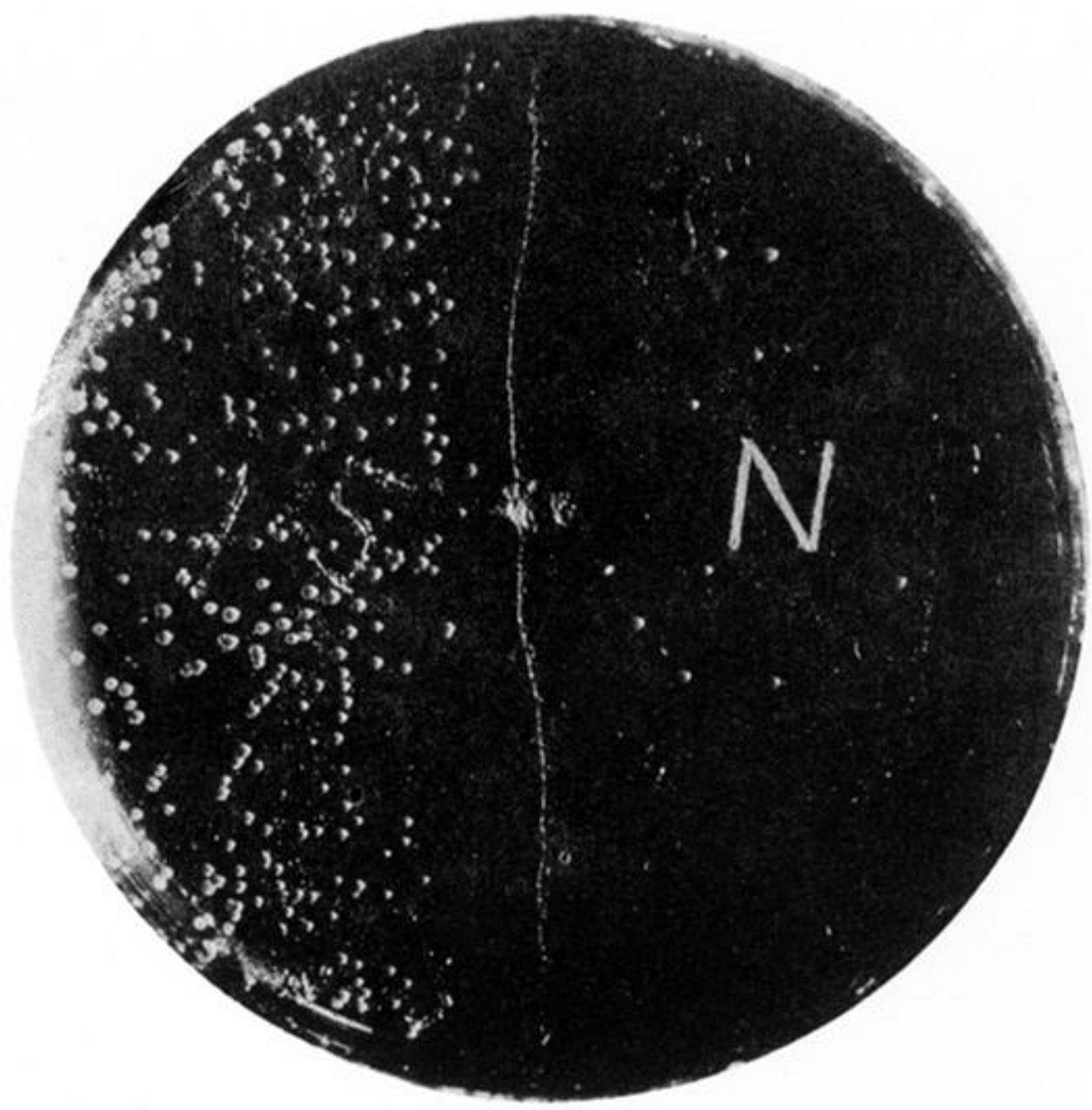


FIG. 1.



1.5% NaCl Sol.

0.85% NaCl Sol.

FIG. 2.

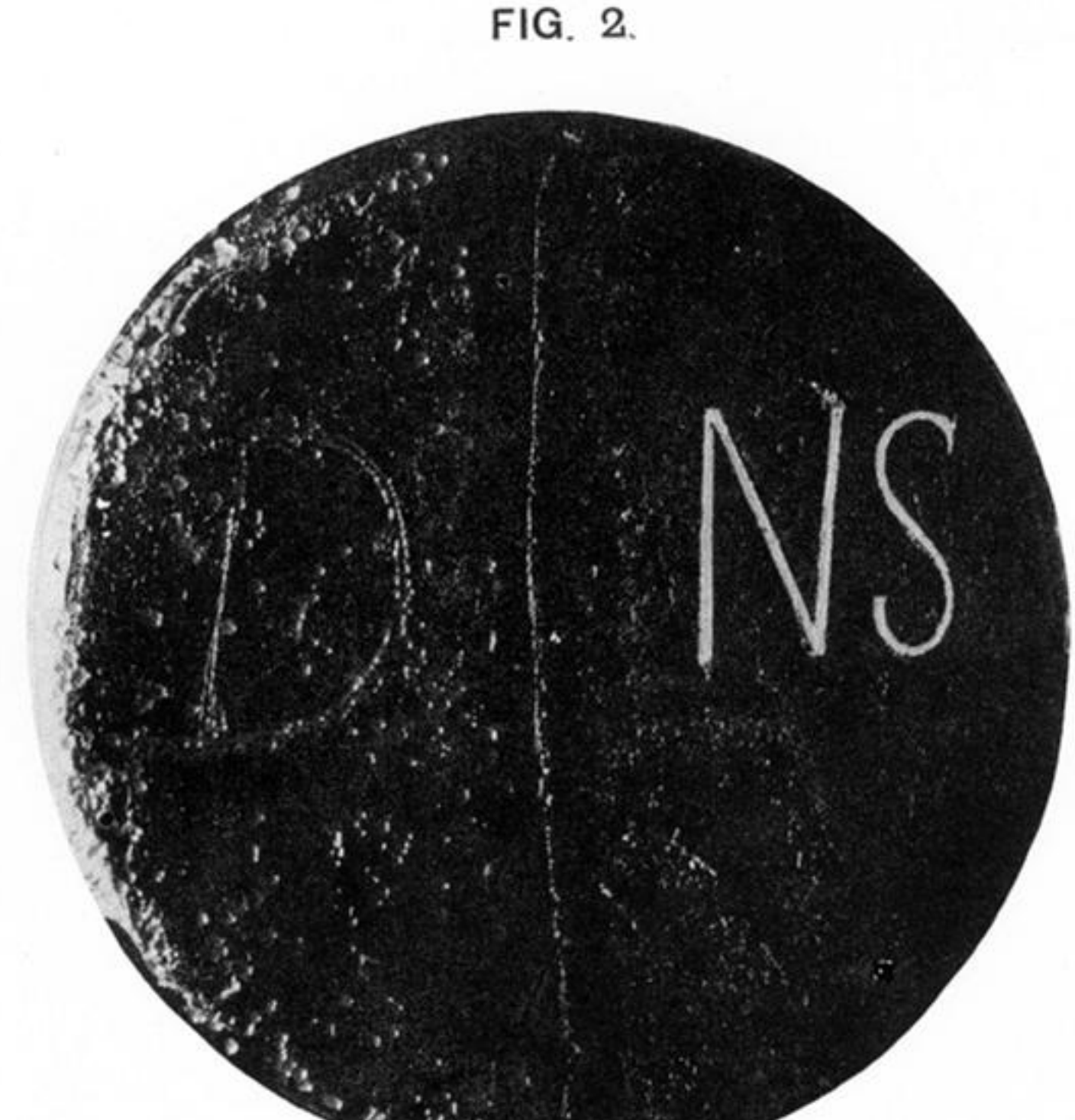


FIG. 3.