features in his series, and contrasts this with the prompt and complete hemiplegia, followed by a fatal issue in 24 to 36 hours, which may occur after ligation of the common carotid artery. In the four cases related, however, the early appearance and completeness of the hemiplegia and the rapidly fatal issue resemble the effects of ligation of the common carotid. It is natural that such cases, only surviving from 15 to 72 hours, would not reach a base hospital.

We are indebted to the Medical Research Committee for permission to make use of the two drawings by Mr. A. K. Maxwell.

ON THE PRESENCE OF AN ACCESSORY FOOD FACTOR IN THE NASAL SECRETION

AND ITS ACTION ON THE GROWTH OF THE MENINGO-
COCCUS AND OTHER PATHOGENIC BACTERIA.

(Preliminary Paper.)

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(Report to the Medical Research Committee.)

1. Introduction.

IN dealing with numbers of cerebro-spinal fever contacts during the recent epidemic it was noticed that the growth of the meningococcus was in some instances strongly influenced by the amount of nasal secretion transferred by the swab to the surface of the culture medium. It was found from experiment that if a trace of dilute sterile nasal secretion was added to the surface of the culture medium, it brought about a rapid growth of the meningococcus when this medium was subsequently inoculated with this germ. The growth is always many times the amount obtained on the same medium, on planting out a similar quantity of culture, in the absence of nasal secretion.¹

The action of sterile dilute nasal secretion in accelerating the growth of the meningococcus would seem to be entirely in the nature of an accessory food body or vitamine, as alone this secretion is quite incapable of supporting or stimulating the growth of this germ. This conclusion is supported by its peculiar properties. It is soluble in water, slightly soluble in alcohol, and almost insoluble in ether.² It has great heat-resisting capacity, withstanding autoclaving at 120° C. and boiling in strong hydrochloric acid for 12 hours without losing strength. On filtration it always undergoes considerable adsorption, even when passed through an ordinary filter-paper, and it almost entirely disappears from any fluid when this is passed through a Doulton filter candle. All chemical manipulation, drying, precipitation, &c., appreciably weaken its power of stimulating the growth of the meningococcus.

In addition to the meningococcus and its allies, it has been shown to accelerate the growth of the pneumococcus, *B. typhosus*, *B. coli communis*, various faecal streptococci, and a number of throat bacteria. It is highly probable that it will accelerate the growth of all pathogenic bacteria when added in sterile fashion to any suitable culture material, whether solid or fluid.³

2. Mode of Collection of Nasal Secretion.

The nasal secretion used in the following experiments was obtained either by collection direct from the naso-pharynx by means of a suction pump, or by the employment of naso-pharyngeal swabs such as those used in the routine swabbing of cerebro-spinal fever contacts. This

latter method proved the most convenient. These swabs are made of ether-washed cotton-wool placed on the ends of flexible wires which are introduced into the naso-pharyngeal region through the mouth retracted within bent glass tubes, so that all contamination from the oral secretion is avoided. The swabs were then extracted in distilled water. It was found that quite a considerable amount of secretion could be obtained in this way. This will be referred to in the following experiments as "dilute" nasal secretion in distinction to that collected "undilute" direct from the mucous membrane of the naso-pharynx, by means of the pump.

3. The Preparation of Various Extracts of Nasal Secretion.⁴

(a) General treatment in the preparation of aqueous, alcoholic, and ethereal extracts. The cotton-wool was removed from the swab wires and covered with the solvent in glass-stoppered specimen tubes of suitable size, using 1-2 c.c. for each swab. After standing for 24 to 48 hours, the liquid was removed with a pipette, and filtered through a 5 c.c. Swedish acid-extracted filter-paper. Aqueous extracts which were further treated, and all alcoholic and ethereal extracts, were evaporated to dryness on a tepid water-bath at a temperature of about 60° C., the basin being removed before it was absolutely dry when water was the solvent, and then taken up in distilled water, 1 c.c. of water being allowed for each swab extracted.

(b) Preparation of hydrolysed nasal secretion. 3 c.c. of undilute nasal slime was heated with ten times its volume of strong HCl in a test-tube standing in boiling water for six hours. More HCl was added to make up for the loss by evaporation, and the heating continued for six hours more. The solution, which was faintly yellow, was transferred to a porcelain dish and the excess of acid was evaporated off on the water-bath with the continual addition of water. When nearly all the acid had been removed the remainder was dried at a low temperature, dissolved in 7 c.c. of water, and made faintly alkaline to litmus paper with N/10 NaOH, about 5 c.c. being necessary.

All the extracts before being sealed up in vaccine tubes were tested and their alkalinity adjusted as closely as possible to Ph. = 7.35 of Sorensen's scale. The extracts after being sealed up were finally sterilised at 55° C. for an hour. All manipulation of the extract was conducted in beakers of Jena glass, or Berlin porcelain or platinum, and great care was taken to have all glassware, especially the slides used for mixing the dilutions, clean and free from any chemicals. The distilled water used was redistilled from glass several times.

4. Method of Testing the Action of Extracts.

The action of the various extracts of nasal secretion in stimulating the growth of the meningococcus and other bacteria was tested by making a number of dilutions of the germ in the extract, and planting out each dilution separately and comparing their growth, with a similar quantity of the same emulsion diluted in a similar manner in distilled water or Ringer's solution, and planted out in the same way. It was found after several trials that distilled water offered the best form of control. In the following experiments care was always taken to make the control dilution first and to plant it out immediately. Thus all harmful action of distilled water on the germ was minimised as much as possible.⁵ Where typhoid and coli organisms were used all emulsions and controls were made in Ringer's solution.

A large number of methods of dilution were tried. The one found most useful consisted in taking up in a calibrated pipette, finely throttled to ensure accurate measurement, nine volumes of the fluid to be tested, each volume representing 25 c.mm. of liquid. They were then spread out separately on sterile slides. Then with a smaller pipette, also throttled, 5 c.mm. of a standard emulsion⁶ of the meningococcus (5000 million to the c.c.) in Ringer's solution, was drawn up. This was added to the first of the 25 c.mm. volumes on the slide and thoroughly mixed by drawing it up to a fixed

¹ If sterile dilute nasal secretion be tested against the action of sterile dilute oral saliva in stimulating the growth of the meningococcus, it will be found that oral secretion has also some power in this respect, but this is much less than with nasal secretion. Gordon has shown (Brit. Med. Jour., June 17th, 1916, p. 849) that in the unsterile state oral saliva has a marked inhibiting action on the growth of this germ. When the various mouth bacteria were removed from this secretion by sterilisation or the centrifuge, this inhibiting action immediately disappeared.

² It can be precipitated with phosphotungstic acid and neutral lead acetate, but this needs further confirmation.

³ In the experiments described in the present paper the medium used is Crowe's "chocolate medium" (see THE LANCET, Nov. 20th, 1915). In other experiments similar results have been obtained with Gordon's legumen tryptagar.

⁴ For the proper preparation of the extracts the Medical Research Committee have kindly placed the services of Mr. D. Matthews at my disposal.

⁵ I have shown elsewhere (THE LANCET, 1916, ii., 902) that the meningococcus is peculiarly resistant to the hypotonic action of distilled water. I have shown that it can withstand its action for many hours without experiencing any apparent ill-effect.

⁶ In speaking of a "standard emulsion" of the meningococcus in the following experiments it will always be understood that one in Ringer's solution of the strength of 5,000,000,000 cocci to the cubic centimetre is meant.

mark each time in the process of mixing. Then from this 5 c. mm. was taken up and transferred to the second 25 c. mm. volume and the mixing repeated. This procedure was gone through in succession with each of the nine volumes. Thus in the end a series of nine successive dilutions of the germ were obtained. Then by means of a fine sterile camel-hair brush or fine capillary tube, a certain quantity of each dilution was transferred in turn to the surface of the culture medium, commencing with the highest and gradually working down to the lowest dilution. The quantity transferred could be accurately measured when a capillary tube was used, but when a brush was employed it was only roughly the same in each instance. The dilutions were spread on the surface of the culture medium by dipping the brush in one of them and then drawing it rapidly across the surface of the plate. This operation was repeated in turn with each of the dilutions. Thus a series of nine parallel lines were obtained on the plate, each representing a particular dilution of the germ in the solution to be tested.⁷

5. Experiments.

*Experiment I.*⁸ (Figs. 1 and 2).—A "standard" emulsion of an old laboratory culture of meningococcus (Littledale) was made up in Ringer's solution. 5 c. mm. of this emulsion was added to 25 c. mm. of distilled water and of an aqueous extract (see under Sec. 3a) of dilute nasal secretion respectively, and diluted down through nine successive dilutions, and planted out by means of a brush as described under Sec. 4. Thus nine parallel lines, as shown in Figs. 1 and 2, were obtained on the plates,⁹ one representing the dilution in nasal secretion extract and the other the control dilution in distilled water. Line No. 1 contains the fewest number of germs, while line 9 contains the largest number. Each line contains very roughly the same number of germs as the corresponding line on the other plate. The plates were incubated at 37° C. for 24 hours and then taken out and the resulting growth photographed. The photographs show the plates reduced to half natural size.

In Fig. 1, showing the dilution in distilled water, little growth has taken place except on lines 7, 8, and 9. In the same degree of dilution in aqueous extract of dilute nasal secretion, Fig. 2, considerable growth has taken place on all the lines, and is confluent in the case of lines 7, 8, and 9. A comparison of the two photographs gives a rough idea of the accelerating action of nasal secretion on the growth of the meningococcus.

It was immaterial whether, in this experiment, Ringer's solution was substituted for distilled water in the control dilutions to obviate all possible harmful action of the distilled water; the result was the same. There is possibly a slight increase in the growth obtained in the control, due to the use of Ringer's solution, but this increase was not sufficient to alter the main result of the experiment. There was always a preponderating growth with nasal secretion extract.

Experiment II. (Figs. 3 and 4).—In this experiment, instead of an old laboratory culture being used as in Experiment I., a freshly isolated strain of the meningococcus (Walters) was taken. This had been obtained from the spinal canal of a cerebro-spinal fever patient about a week previous. In this instance the dilutions of a "standard" emulsion were made as before in distilled water and nasal secretion (aqueous extract), but instead of a small quantity of each dilution being transferred to the plates by means of a brush, each dilution containing 30 c. mm. of fluid was transferred by means of a fine capillary pipette to the surface of the culture medium. Thus, instead of a series of lines, a series of drops or blebs were obtained representing 11 consecutive

dilutions of the germ in distilled water and nasal extract respectively.

A comparison of Figs. 3 and 4 shows the accelerating action of the nasal secretion extract over that of the distilled water one. The figures, however, hardly bring out the proper difference between them, as the spreading of the drops on the surface of the medium has been irregular. Actual inspection of the plate, Fig. 4, shows, for instance, that dilution No. 11, which contains the fewest number of organisms, is very thin and spread out, while No. 1, which contains the largest number of germs, is very heaped up and thick, a condition which is not very obvious from the photograph.

The comparison of dilutions 7-11 of Fig. 4 with the same dilutions in Fig. 3 dispels any doubts as to the accelerating action again of nasal secretion in this experiment.

Experiment III.—In this experiment it was sought to find out the amount of accelerating action nasal secretion extract brought about on the growth of the meningococcus when growing in a fluid broth medium and not a solid medium as in the former experiments. To two small vaccine tubes containing each 2 c.c. of sterile glucose litmus broth 5 c. mm. of "standard" emulsion of meningococci (Walters) was added. To one of the tubes in addition 5 c. mm. of an aqueous extract of dilute nasal secretion was added. The tubes were then incubated for 48 hours at 37° C. At the end of this time the tube that had received the nasal secretion in addition to the meningococci had entirely lost all trace of pigment, while that which was without the extract still retained a certain amount of reddened litmus pigment. The tubes were then carefully shaken up to ensure an even distribution of the cocci, and counts were made of a certain volume of fluid from each tube. This showed that there was roughly eight times the number of cocci per c. mm. in the tube with the nasal extract as compared with that without it.

Experiment IV.—In this experiment an attempt was made to see if prolonged boiling of aqueous extract of dilute nasal secretion had any action in destroying its power to stimulate the growth of the meningococcus. 4 c.c. of an aqueous extract of dilute nasal secretion was boiled continuously for five hours, distilled water being added from time to time to keep it at the same volume. (See Sec. 3 b.) It was then tried out as before, against some of the same aqueous extract unboiled, with a "standard" emulsion of meningococcus (Littledale). It was found that the boiling had had but little or no effect in weakening the action of the nasal secretion.

Experiment V. (Figs. 5, 6, and 7)—In this experiment hydrolysed nasal secretion prepared by boiling undilute nasal slime with strong hydrochloric acid for 12 hours (as described in Sec. 3 (b)) was tested against the action of the same nasal secretion untreated with HCl. Dilutions of a "standard" emulsion of a freshly isolated culture of meningococcus (Lake) were made in distilled water (control), nasal slime, and nasal slime hydrolysed, respectively. These were planted out on a plate by means of a brush in the usual way.

Photographs 5-7 show the result of this experiment. It will be seen that the hydrolysed nasal secretion is as active in accelerating the growth of the meningococcus as the same secretion untreated (Figs. 6 and 7). The action of both hydrolysed and the non-hydrolysed secretion in accelerating growth is well shown by comparison of these plates with that planted out with the control dilutions in distilled water. This experiment has been repeated a number of times with different strains of meningococci and always gave the same result.

Experiment VI. (Figs. 8-11).—To determine the relative power of a plain alcoholic extract of dilute nasal secretion, as compared with a plain aqueous extract and an ether extract of the same in stimulating the growth of the meningococcus. Dilutions of a standard emulsion of meningococcus (Paul) were made as before. The diluents were distilled water (control), aqueous extract of dilute nasal secretion, plain alcoholic extract of dilute nasal secretion, and ether extract of the same. Figs. 8-11 show the resulting growth obtained after 24 hours' incubation at 37° C. Comparison of Fig. 10 with Fig. 9 shows the action of a plain alcoholic extract of dilute nasal secretion to be almost as active as that of an aqueous one, while comparison of both these figures, with the control in distilled water (Fig. 8) shows the amount of acceleration in growth produced.

The conclusion drawn from this experiment was that the active principle in nasal secretion which accelerates the

⁷ If we commence with an emulsion of 5000 million meningococci to a cubic centimetre, and make nine consecutive dilutions of 5 parts in 30 in each dilution, then theoretically we should expect to find in each of the nine dilutions the number of cocci per cubic centimetre as follows:—

Dilution.	Cocci.	Dilution.	Cocci.
1	833,000,000	6	170,000
2	139,000,000	7	18,000
3	23,000,000	8	3,000
4	4,000,000	9	500
5	640,000		

These numbers were never actually approached in the following experiments, as the pipettes and brushes were not sterilised in passing from one dilution to another.

⁸ The numbering of the experiments is only for the convenience of the present paper; many of the experiments described have been repeated a great many times.

⁹ All the experiments described in this paper refer to implantations on Crowe's "chocolate medium."

EXPERIMENT I.—*Meningococcus* "Littledale."

FIG. 1.—Dilutions in distilled water. (Control.)
FIG. 2.—Dilutions in aqueous extract of sterile dilute nasal secretion.

EXPERIMENT II.—*Meningococcus* "Walters."

FIG. 3.—Dilutions in Ringer's solution.
FIG. 4.—Dilutions in dilute aqueous extract of nasal secretion.

EXPERIMENT VI.—*Meningococcus* "Paul."

FIG. 8.—Dilutions in distilled water.
FIG. 9.—Dilutions in aqueous extract of nasal secretion.

FIG. 10.—Dilutions in alcoholic extract of nasal secretion.
FIG. 11.—Dilutions in ether extract of nasal secretion.

EXPERIMENT V.—*Meningococcus* "Lake."

FIG. 5.—Dilution in distilled water. (Control.)

FIG. 6.—Dilution in aqueous extract of dilute nasal secretion.

FIG. 7.—Dilution in hydrolysed nasal secretion.

EXPERIMENT VII.—*B. typhosus*.

FIG. 12.—Showing the action of dilute nasal secretion in stimulating the growth of *B. typhosus*. The upper half of the plate shows the dilutions of the germ in Ringer's solution, while the lower half shows the dilutions in dilute aqueous extract of nasal secretion.

growth of the meningococcus is soluble in distilled water, less so in alcohol, and almost insoluble in ether.

Experiment VII. (Fig. 12).—To determine the action of nasal secretion extract on the growth of *B. typhosus*. In Fig. 12 the upper half of the plate has been planted out in the usual way with dilutions of an emulsion of *B. typhosus* in Ringer's solution; in the lower half, with dilutions of the same emulsion in aqueous extract of dilute nasal secretion. Fig. 12 shows that a much more abundant growth took place on that half of the plate which received the nasal secretion.

The conclusion drawn from this experiment was that the growth of *B. typhosus* is accelerated in a similar manner to that of the meningococcus by an aqueous extract of dilute nasal secretion.

Similar results were obtained in experiments with the following germs:—*Streptococcus zymogenes*, *Micrococcus pharyngeus flavus*, pneumococcus, and *B. coli communis*.

Conclusion.

The outstanding results of the foregoing experiments show that in nasal secretion there is present some body which greatly accelerates the growth of the meningococcus on an artificial culture medium. Alone it is incapable of acting as a food or stimulant to the growth of this germ. It is soluble in water, less so in alcohol, and very insoluble in ether. It has great heat-resisting power, being able to resist prolonged boiling for many hours. It is not destroyed by boiling in the presence of strong hydrochloric acid for twelve hours. In addition to the meningococcus it also stimulates the growth of many other pathogenic germs.

A NOTE ON THE CAUSE AND PREVENTION OF TRENCH FOOT.

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No one who has had an opportunity of observing many cases of trench foot, particularly in the later stages of the disease, can have failed to be struck with certain characteristics presented by it. The lesions are indolent, slow to heal, and prone to break down again on the slightest provocation. The scar tissue formed is weak and ulcerates easily. Indeed, the whole picture of the disease reminds one irresistibly of the trophic sores appertaining to diseases of the central nervous system. I wish to set forth in this paper my reasons for thinking that trench foot is an affection of a trophic nature.

Some three years ago a book on electro-pathology was published by Mr. A. E. Baines, a distinguished electrical engineer. Space does not permit me to give an account of the far-reaching vista of promise which this little-known work has opened up to medicine. An abler pen than mine has recently put the whole matter before the profession. Let it suffice to say that Mr. Baines believes that the brain, and possibly the cord, is the seat of electrical generation, and that the current so generated is carried to the tissues by means of the nerves, which to the electrician's view are nothing but insulated cables. Here we have the trophic influence of physiology and medicine, that vague generalisation which from our student days we have been content to accept without questioning. Mr. Baines has shown us how to measure and modify this trophic influence, and I venture to predict that in the future his name and fame will equal that of those illustrious ones, Simpson, Lister, and Koch, as benefactors of the human race. This is no mere discovery of an isolated fact in medicine. Many a patient worker has given his brick, his hard-wrought stone of research to the building of the sacred fane of Æsculapius. As is perhaps fitting and proper for an engineer, Mr. Baines has come and shed a flood of light in our temple. For he has given us a fundamental principle.

The dominating factor in the conditions under which trench foot occurs is wet. When a man is standing for days on end in water and liquid mud, his boots, socks, and the skin of his feet and legs become soaked. As is well known, the electrical resistance of the skin is enormously diminished when the skin is wet. Under these conditions a gradual leakage of electricity to earth takes place from the feet. Other things being equal, electricity will always leak

from a point in preference to a plane surface, which accounts for the fact that the toes are most affected. This leakage deprives the cells of the foot tissues of their trophic influence and they die. Hence the lesions of trench foot.

Local leaks of electricity may be detected by a suitable galvanometer. One pole of the galvanometer should be connected to a silver plate about the size of a florin, which is fixed in the centre of the patient's forehead; there is no special reason for fixing it on the forehead except that it is the most convenient spot in the middle line of the body. The other pole is connected to a contact plate mounted in an insulated handle so that the contact or electrode can be placed on any portion of the body it is desired to test. Before testing it is necessary to "earth" the patient in order to dissipate any induced charge of electricity of which he may be the host. If this is not done the patient's own intrinsic current may be swamped by charges he has picked up from trams, electric trains, or other sources. With both poles in contact with the body the galvanometer will give a deflection. When leakage is taking place from any particular area a high and rapid deflection is noted. I have been examining and treating various cases on Baines's principles for a matter of about two years, and have found such a leakage taking place from the feet and legs of soldiers suffering from trench foot whom I had under my care last spring. Moreover, the results I obtained by treating these cases by dielectric oil were better than the results obtained by any other method I tried. I also made a few experiments bearing on this subject.

Experiment 1.—With the aid of Miss Flecker, at the Ladies' College Physical Laboratory, Cheltenham, I estimated the electrical resistance of a piece of oak tanned sole leather 3 inches long by 1 inch wide. We found that when dry the resistance was practically infinity. When wet the resistance is that of the fluid the leather has soaked in.

Experiment 2.—One pole of the galvanometer was connected to an electrode which could be held in the hand. The other pole was connected by an insulated cable to a copper plate imbedded in the earth. Another insulated cable was connected at one end to the metal pipe supplying water to the house, and at the other end to a brass rod of 1 inch section. After earthing myself I held the brass rod in one hand and the electrode in the other, and obtained a rapid off-scale deflection, showing, firstly, that an electric current was coming from my body; and secondly, that the earth connexions were working properly, for the current passed out by one hand through the brass tube to the water pipe, thence about 20 feet through the earth to the copper plate, and through the galvanometer to the other hand, so completing the circuit.

Experiment 3.—The brass tube was then laid on the floor which was covered by a thick carpet. I held the electrode by one hand and put both feet on the brass tube. I wore ordinary boots which were dry. No deflection was obtained because the dry leather soles of my boots insulated me from the earth. I then took my boots off and put my bare feet on the tube and obtained an off-scale deflection.

Experiment 4.—Next day was wet and I walked about half a mile so that the soles of my boots, which were free from holes and metal nails, became wet. On holding the electrode in one hand and placing my feet on the brass tube, a rapid off-scale deflection occurred, showing that current was passing through my boots to earth.

Experiment 5.—The pole of the galvanometer connected to earth by the copper plate was disconnected. It was reconnected to a hand electrode exactly like the one previously used, so that the galvanometer was now connected to the hand electrodes only. After the necessary earthing process, I held the electrodes in the hands and obtained a deflection which remained steady at 170 mm. I then placed my feet, still in wet boots, on the brass tube and awaited results. The light on the scale very slowly began to recede towards zero. I repeated this experiment several times. The light never remained at zero, but if it got as far went over to the other side of the scale, and generally registered 40 to 60 mm. I take this as evidence that electricity was gradually leaking out of my body to earth, through my wet feet. One would not expect the light to register zero as there is continuous generation of electricity in the body. In view of these experiments, the grandmotherly advice we have so often received, not to stand about in wet boots, takes on a new and important significance which ought to claim our belated respect. They also to my mind afford evidence that trench foot is probably caused by long-continued leakage of electricity from the feet.

I noted that Dr. Leonard Hill had advocated rubber boots and greasing of the feet for the prevention of the disorder, recommendations with which I cordially agree. I sent to him in the spring a note about my own experiments, and