

STUDIES ON THE NASOPHARYNGEAL SECRETIONS FROM PATIENTS WITH COMMON COLDS.

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The experimental studies on the incitant of common colds in man to be reported in this paper were begun shortly after the investigations on influenza by Olitsky and Gates¹⁻³ were undertaken. They were initiated by the desire to check or control the latter studies and to determine, if possible, any causative agent in the nasopharyngeal secretions derived from persons suffering from common colds. This work has now extended over 4 years, and, while still incomplete, our purpose is to state only the results which we regard as established experimentally.

Source of Materials.

It is obvious that patients subjected to study should be selected carefully, for the pathognomonic symptom, rhinitis, can be induced by exposure to cold and by other physical and chemical agents, and is a sign of several affections, such as hay-fever, influenza, measles, and other exanthemata. Only such affected individuals were chosen who could trace their illness to exposure to a previous case of common cold within 3 or 4 days; in other words, only those who had an infectious condition and showed at the same time the typical syndrome of the disease. This consists of premonitory malaise and dryness of the nasal passages, followed by sneezing and discharge of a profuse, thin, irritating secretion from the nose. Conjunctivitis of a mild degree with

¹ Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1921, xxxiii, 125, 361, 373, 713; xxxiv, 1; 1922, xxxv, 1, 553, 813.

² Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxvi, 501.

³ Olitsky, P. K., and Gates, F. L., *J. Exp. Med.*, 1922, xxxvi, 685; 1923, xxxvii, 303; *Science*, 1923, lvii, 159.

lacrimation is usually present and in some persons slight headache. The temperature is either not elevated or only slightly so and in general the constitutional reaction is negligible. On the 2nd or 3rd day the nasal discharge diminishes in quantity, becomes first mucoïd and then purulent; the mild constitutional symptoms, if present at the beginning, disappear, and in uncomplicated cases, complete recovery ensues after 4 or 5 days. In the purulent stage, however, secondary localizations may occur by extension of the inflammation to the ear, inducing otitis media, or to the paranasal sinuses, or to the larynx. In these instances, the microorganisms usually found associated are the staphylococcus, streptococcus, pneumococcus, and Friedländer bacillus groups, the meningococcus, *Micrococcus catarrhalis*, and Pfeiffer's bacillus.^{4,5}

Ringer's or 0.85 per cent saline solutions were employed to wash the nasopharyngeal secretions from cases of common colds. These washings were collected from 40 patients in the early hours (from 3 to 20) of the uncomplicated illness. In addition, washings were obtained from 30 individuals, supposedly free from common colds, and were employed as controls. In general the methods used in the following experiments were those described in the series of papers on influenza by Olitsky and Gates.¹⁻³

Transmission Experiments.

Common cold is a mild disease in man; constitutional reaction is either lacking or is transitory and inconsequential. There is no definite, measurable criterion of a positive transmission to rabbits—the animals employed in the experiments. The symptoms of nasal discharge and sneezing cannot be regarded as critical in rabbits, for these animals, as a future report will show, develop snuffles spontaneously in association at times with an underlying chronic paranasal sinusitis. Thus, after nasal swabbing of thirteen rabbits with filtered and unfiltered nasopharyngeal washings from fresh cases of common cold in man, six showed, within 2 to 4 days thereafter, symptoms of nasal

⁴ Lacy, G. R., *J. Lab. and Clin. Med.*, 1918, iv, 55.

⁵ Crowe, S. J., and Thacker-Neville, W. S., *Bull. Johns. Hopkins Hosp.*, 1919, xxx, 322.

discharge, a proportion which prevailed, however, among an equal number of animals swabbed with control materials. Similarly, the intratracheal injection into fifteen rabbits of the unfiltered nasopharyngeal washings derived from ten cases of early common colds was followed by no constant or characteristic effects. Although the lung tissue of injected rabbits was reinoculated into two successive series of animals, the results of these intratracheal inoculations showed that some animals gave a polynucleosis with frank lobar pneumonia, from the lungs of which either the pneumococcus or streptococcus or rabbit septicemia bacillus could be recovered in pure culture, others a mononucleosis without lung involvement, and the remainder, inconstant effects. Similar results were obtained from the washings of six supposedly normal individuals injected intratracheally into eleven rabbits.

The conclusions to be drawn from our attempts at transmission to rabbits are that, in the absence of a suitable criterion for a positive transmission or because of the mildness of the disease, these animals failed to show any definite effects. On the other hand, it is apparent that experimentally the nasopharyngeal secretions of early cases of common colds are distinct from those of influenza, since the latter, as already demonstrated,¹⁻³ induce constant and striking effects both in the blood and on the lungs of rabbits.

Transmission to Man.

It is apparent that a disease of so mild and transitory a nature as the common cold could best be studied, in as far as transmission of any possible incitant is concerned, in man. Before describing the experiments the previous work of Kruse,⁶ in 1914, and Foster,⁷ in 1915-16, should be mentioned.

Kruse diluted the nasal secretion of an assistant ill with a common cold with 15 times its volume of saline solution and then filtered the material through a Berkefeld candle. A few drops of the filtrate were instilled into the nose of each of twelve men. In 1 to 3 days, four of them showed common colds. On repetition, the nasal discharge from a case of common cold was taken up in 20 volumes of saline solution and instilled into the nose of each of 36 volunteers. In 1 to 4 days

⁶ Kruse, W., *Munch. med. Woch.*, 1914, lxi, 1547.

⁷ Foster, G. B., Jr., *J. Infect. Dis.*, 1917, xxi, 451; *J. Am. Med. Assn.*, 1916, lxi, 1180.

fifteen, or 42 per cent, became ill with this disease. Kruse concluded that the incitant of common colds is a filterable microorganism, although he was unable to demonstrate either morphologically or by culture a formed, living and multiplying element.

Foster later believed that he confirmed the findings of Kruse. The nasal secretions from cases of common colds within 24 hours after the onset of the first symptoms were suspended in 10 cc. of saline solution and, after shaking, the homogeneous suspension was filtered through a Berkefeld N candle. From 3 to 6 drops of the filtrate were instilled into both sides of the nose in each of ten men. "7 developed clear cut and definite symptoms of acute coryza; 2 reacted questionably, while 1 remaining case exhibited no symptoms. There was an incubation period of 6 or 8-30 hours. . . . The duration of the symptoms varied 3-6 days—usually 5." In addition, Foster stated that he has cultivated in fresh tissue—ascitic fluid medium from eleven cases of the natural disease and from five of experimental common colds a minute anaerobic microorganism similar in many respects to the "globoid bodies" of Flexner and Noguchi.⁸

The present experiments were made on nineteen volunteers with the washings collected from eight patients. The mode of procedure differed somewhat from that of Kruse and Foster. Instead of the nasal discharges, we obtained the nasopharyngeal secretions by washing with 25 to 30 cc. of either Ringer's or 0.85 per cent saline solutions.¹⁻³ The suspension was shaken and then filtered through a Berkefeld V or N candle. The clear filtrate free from ordinary bacteria, as determined by cultivation tests, was applied to cotton applicators 2 cm. long and 0.5 cm. wide, the cotton being thoroughly soaked, and then conveyed to the nasal mucous membrane, on each side, by gentle swabbing. In each series of experiments one or two volunteers were swabbed with the unheated filtrate and another, as a control, with the same material but heated at 60°C. for 1 hour. Furthermore, the cases were earlier in the course of the illness than those reported by Foster; namely, from 3 to 20 hours after the onset of the first symptoms.

Experiments on transmission from man to man were attempted with the filtered nasopharyngeal secretions from six patients in the early stages of infectious common colds. The following protocols are illustrative of the tests.

⁸ Flexner, S., and Noguchi, H., *J. Exp. Med.*, 1913, xviii, 461.

Protocol 1. Patient A.—Exposed to a case of a common cold 3 days previous to onset of symptoms. Illness characterized by a profuse, serous, irritating discharge from nose, slight conjunctivitis, but practically no fever or constitutional symptoms. This lasted for 2 days, after which the patient returned to normal. The nasopharyngeal secretions were collected in 25 cc. of Ringer's solution, 3 hours after the onset of the first signs of the affection. The unfiltered washings on aerobic culture showed growths of *Staphylococcus albus* and Pfeiffer's bacillus. The filtered washings on aerobic blood agar plate cultures yielded no growth. Anaerobic cultures of this and the following patients to be mentioned will be described later.

The filtrate was separated into two portions: one, unheated, was applied by swabbing to the nasal mucosa of Volunteer 1; the other, heated at 60°C. for 1 hour, to that of Volunteer 2. Volunteer 2 was unaffected.

Volunteer 1, in the afternoon of the 2nd day after swabbing, complained of malaise and dryness of the nose. The next morning he presented a profuse, thin serous discharge from the nose, felt feverish, and suffered from headache. The following day the symptoms were aggravated, and the nasal discharge, which was profuse and mucoid, became purulent on the 4th day of the illness. During the 5th to 7th days the affection subsided; the constitutional symptoms disappeared and only a slight purulent discharge was noted; but on the 8th day a right maxillary antrum infection supervened which endured for 2 weeks.

18 hours after the onset of the first symptom, the nasopharyngeal secretions of this person were collected in 25 cc. of Ringer's solution. The unfiltered washings showed on aerobic culture an occasional colony of *Staphylococcus albus* and *Streptococcus viridans*; the filtered washings yielded no growth.

The filtrate was treated as in the case of Patient A and applied, unheated, to the nasal mucosa of Volunteer 3 and heated on that of Volunteer 4. The latter was unaffected. Volunteer 3, who is ordinarily resistant to common colds, showed after 24 hours a profuse, thin discharge from the nose without any other symptoms. This lasted for only 1 day, after which the discharge disappeared.

Protocol 2. Patient B.—Exposed to a case of a common cold 2 days previous to onset of symptoms. There were no constitutional symptoms in this individual, only a profuse serous and later mucoid discharge from the nose and lacrimation, which endured for 2 days.

12 hours after the onset, the nasopharyngeal secretions were collected in 25 cc. of Ringer's solution. The unfiltered secretions yielded an occasional colony of *Staphylococcus albus*, Pfeiffer's bacillus, and diphtheroids on aerobic culture; the filtered material exhibited no growth.

Volunteer 5 was swabbed nasally with the unheated filtrate, Volunteer 6 with the heated. The latter was not affected. Volunteer 5, 8 hours after swabbing, felt an increase of mucus in the nasopharynx. On the next day there was a profuse, thin, serous secretion from the nasal mucosa but without constitutional disturbance. On the 2nd day the discharge became mucoid and the nose was clogged so that he was forced to breathe through the mouth. On the 3rd day the

nasal secretions were profuse and purulent. The following day saw improvement in his condition, but on the 5th day there was an exacerbation of the acute attack. The duration of illness was 6 to 7 days.

20 hours after the onset of the affection the nasopharyngeal secretions of Volunteer 5 were collected in 30 cc. of Ringer's solution. The aerobic cultures of the unfiltered washings gave a few colonies of *Micrococcus flavus* and *Streptococcus viridans*; those of the filtrate were sterile.

The heated filtrate was swabbed on the nasal mucosa of Volunteer 7, who was not affected, and the unheated filtrate on that of Volunteer 8, who exhibited 8 hours later dryness of the nasal mucous membrane; 17 hours later there was an increased thin discharge without lacrimation or constitutional disturbance. 24 to 48 hours later the discharge was thicker, mucoid, and profuse, and on the 3rd day the subject was well.

Protocol 3. Patient C.—Two associates of this woman, including herself, became ill with common colds within 2 to 3 days of each other. 5 days after the onset of symptoms, her mother became ill with this disease. Patient C suffered from a mild attack; the 1st day was characterized by a profuse serous nasal discharge, the 2nd by a change in the nature of the secretions to mucoid and the 3rd day to mucopurulent. Except for lacrimation there were no symptoms.

6 hours after the onset, the nasopharyngeal secretions were washed with 30 cc. of Ringer's solution. The unfiltered washings yielded on aerobic culture numerous colonies of *Staphylococcus albus*; the filtered, no growth.

The filtrate was swabbed on the nasal mucosa of Volunteer 9. 14 hours later sneezing and dryness of nasal mucous membrane were noted. The next day this person showed injected conjunctivæ with lacrimation, sneezing and dryness of nasal mucosa, slight headache, and malaise. The following day there was a thin, serous discharge from the nose and slight laryngitis. The nasal symptoms and the laryngitis lasted for a day; thereafter the subject gradually returned to normal.

The nasopharyngeal washings from this volunteer were obtained 48 hours after the onset. The unfiltered material showed on aerobic culture a few colonies of *Micrococcus flavus* and *Streptococcus viridans*; similar tests on the filtrate were negative. The filtrate was swabbed on the nasal mucosa of Volunteer 10 who was not affected.

Protocol 4. Patient D.—This patient was in contact with two cases of common colds, one a week, the other 3 days previously. He had a profuse, thin, irritating, nasal discharge with excoriated skin about the nose. This condition endured for 3 days.

18 hours after the onset of symptoms, the nasopharyngeal secretions were collected by washing with 25 cc. of Ringer's solution. The unfiltered washings yielded a pure culture of aerobic diphtheroid bacilli; the filtered material was free from aerobic bacteria.

The heated filtrate was applied to the nasal mucosa of Volunteer 11, who remained normal. The unheated filtrate was swabbed on the nasal mucous membrane of Volunteer 12, who 24 hours later exhibited a profuse, mucoid, nasal dis-

charge with collection of mucus in the nasopharynx. 48 hours later this person had a profuse, mucopurulent, nasal discharge and perspired freely (a symptom ordinarily concomitant with common colds in this man's experience). The symptoms disappeared 2 days later.

No attempts at further transmission were made in this instance.

Protocol 5. Patient E.—Contact with common cold case 2 days previous to onset of symptoms which were typical of those of a common cold. The nasopharynx was washed with 25 cc. of Ringer's solution 18 hours in the course of the affection. The unfiltered washings yielded Pfeiffer's bacillus, *Staphylococcus aureus*, and *Streptococcus viridans*; the filtered washings were free from aerobic bacteria.

The heated filtrate was swabbed on the nasal mucosa of Volunteer 12 and the unheated on that of Volunteers 13 and 14. None of these persons was affected.

Protocol 6. Patient F.—This patient was exposed to three others with common colds 3 days before the onset of his illness. The attack was typical and endured for 4 days. 20 hours after the onset of symptoms, the nasopharyngeal secretions were collected in 30 cc. of Ringer's solution. The unfiltered washings exhibited a few colonies of *Staphylococcus albus* and *Streptococcus viridans* on aerobic plate cultures; the filtered washings were free from growth.

The heated filtrate was applied to the nasal mucosa of Volunteer 15 and the unheated to that of Volunteers 16 and 17. All remained normal.

The experiments, although limited in number indicate the transmission of a condition similar to common colds from man to man with the filtered nasopharyngeal washings derived from four of six patients in the early hours of the disease. The two patients with whom transmission failed were 18 and 20 hours in the course of a common cold; the four from whom positive results were obtained were ill for 3, 6, 12, and 18 hours when the tests were made. In two of these four cases, transmission was effected to a second person; in one, such transmission failed, and in the fourth no attempt was made beyond the first passage. Altogether, therefore, definite symptoms were induced in six persons, of whom one developed a secondary maxillary antrum infection and the other a secondary laryngitis. Of the six persons, the first symptoms were noted 8 hours after swabbing in two cases, 24 hours in two, and 14 and 48 hours in the fifth and sixth individuals.

Control Experiments.

In the foregoing experiments it will be noted that the filtrates of the nasopharyngeal washings from early cases of common colds,

heated for 1 hour at 60°C., failed to produce any effects in seven individuals. It seemed, however, that if parallel experiments should be made with the filtrates derived from cases regarded as suffering from non-infectious "colds," the evidence to be deduced from the control series would be strengthened.

Protocol 7. Patient G.—There was no contact with a case of a common cold but a definite history of exposure to cold and wet weather. Since then the patient had had a running nose without other manifestations, which lasted for 2 days.

12 hours after the onset, the nasopharyngeal secretions were washed with 25 cc. of Ringer's solution. The washings, which showed on aerobic culture *Streptococcus viridans* and Pfeiffer's bacillus, were filtered. The filtrate, free from aerobic bacteria, was swabbed on the nasal mucosa of Volunteer 18, who remained unaffected.

Protocol 8. Patient H.—There was no contact with a case of a common cold. Patient was exposed to continuous cold weather in an all day automobile ride. A thin discharge from the nose occurred on the next day and continued until the following noon. The nasopharyngeal secretions were collected in the first 3 to 4 hours after the onset in 25 cc. of Ringer's solution. Aerobic plate cultures yielded five colonies of *Staphylococcus aureus* from the unfiltered washings but no growth from the filtered material. The filtrate was swabbed on the nasal mucous membrane of Volunteer 19, who was not affected.

These two persons who evidently had non-infectious colds did not induce the transmission effects in inoculated individuals.

Cultivation Experiments.

We have already indicated, in the foregoing protocols, that cultures by ordinary means, such as by the employment of rabbit blood agar plates or veal infusion broth under aerobic conditions, did not yield growths of any constant or distinctively pathogenic microorganisms from either the nasopharyngeal washings or from rabbit lungs inoculated with these materials. The results with anaerobic methods will now be described.

The filtered nasopharyngeal secretions of 40 patients with early, typical, infectious common colds, and the unfiltered lung tissue suspensions of fifteen rabbits injected intratracheally with the secretions from ten of the patients, were cultured in the Smith-Noguchi, fresh tissue-ascitic fluid medium under a petrolatum seal. In none of the cultures was any predominating, pathogenic microorganism seen. It

is noteworthy that in no instance was *Bacterium pneumosintes* found. In this regard, the nasopharyngeal secretions derived from common colds differ from those obtained in influenza, since this bacterium has been shown to bear a definite relationship to influenza.¹⁻³ We were also unable to cultivate the so-called "globoid bodies" of Foster.⁷ The appearance of precipitate in this medium simulating morphologically definite bodies will be discussed below.

Later in the course of the cultivation experiments the anaerobic plate method was also used. This has already been described¹⁻³ and consists of 5 per cent rabbit blood plain agar in ordinary Petri dishes placed in the Brown anaerobic jar.⁹ The advantages of the anaerobic plates in conjunction with the Smith-Noguchi fluid medium have been demonstrated in connection with the cultivation experiments with influenzal materials.²

The combined method of Smith-Noguchi medium and the anaerobic plates was employed in the cultivation of the filtered nasopharyngeal washings from nineteen of the forty cases mentioned. This procedure also failed to reveal a constant, definite, pathogenic agent, or Foster's globoid bodies. By means of the anaerobic plates, however, a number of microorganisms were isolated belonging to the three groups of anaerobic, filter-passing, Gram-negative bacteria described by Olitsky and Gates.²

Representatives of the first group of these microorganisms which are actively motile, spirochete-like, slender, curved bacilli, were obtained in eight of the nineteen cases studied. In two of the eight, bacteria of the other two groups were also found; in one, those of the second group and in the other case those of the third group. In a parallel series of experiments with six supposedly healthy individuals and six patients in the early hours of epidemic influenza, similar microorganisms were isolated in three of the normal controls and in two of the influenza cases. Of the latter two, in one instance bacteria of the third group were also obtained. In addition, this type of organism was recovered from a patient suffering from follicular tonsillitis.

The second group consists of minute, tenuous, pleomorphic bacilli. A representative of this group was obtained in one of the nineteen

⁹ Brown, J. H., *J. Exp. Med.*, 1921, xxxiii, 677; 1922, xxxv, 467.

cases of common colds in the culture of which bacteria of Group I were also noted. The same species was cultivated from the nasopharyngeal secretions of one of two cases of acute follicular tonsillitis but not from those of nine supposedly healthy subjects or from the six cases of influenza.

The third group comprises bacteria of actively motile, very tenuous, slender, vibrio-like microorganisms which are smaller than those of the first group and are characterized by their failure to grow in fluid medium. Bacteria of this class were found in three of the nineteen cases of common colds, and in one of the three were associated with microorganisms of the first group. The same species was isolated in four of nine supposedly healthy individuals and in one of six cases of influenza.

Additional serological tests to those already reported² indicate that the rabbit immune serum produced by repeated injections of each type of these microorganisms shows each group to be distinct from the others; there is no cross-agglutination among the different types. Furthermore, these rabbit immune sera fail to agglutinate *Bacterium pneumosintes* and conversely, anti-*pneumosintes* immune serum does not agglutinate any of the microorganisms in the three groups.

Live cultures of fourteen strains of these "group" bacteria were injected intratracheally in rabbits to test their pathogenicity. None of the rabbits exhibited distinctive changes either in the blood or lungs or temperature. In only two instances were bacteria recovered from the lungs of the rabbits similar to those inoculated. Both of these microorganisms belonged to the first group. In addition to the rabbit tests for pathogenicity, a culture of each type was swabbed on the nasal mucosa of each of three persons. None was affected.

To sum up the cultivation experiments with the filtered nasopharyngeal secretions from early cases of typical infectious common colds, it appears that no single, constant, pathogenic agent was found in aerobic or anaerobic media. By means of a combination of Smith-Noguchi medium and anaerobic rabbit blood agar plates, however, a number of microorganisms belonging to the three groups already described by Olitsky and Gates² were isolated. Their irregularity of occurrence in common colds, influenza, and supposedly normal individuals, their differentiation into three distinct species, and their lack of

pathogenicity for rabbits or man would indicate that they are not the incitants of common colds. Finally, it is to be emphasized that *Bacterium pneumosintes* was not found in any of the cultures from the nasopharyngeal secretions of common colds nor from those of the supposedly normal persons employed as controls.

DISCUSSION AND SUMMARY.

From the foregoing experiments it appears that with the filtered nasopharyngeal secretions from early cases of typical infectious common colds in the first 3 to 18 hours of the disease, a similar condition can be transmitted to man. With the unheated but not with the heated secretions from four of six such patients we have succeeded in transmitting an affection indistinguishable from common cold to four men and in two instances the condition was conveyed from the person with the experimental disease to a second individual—in all, therefore, to six supposedly normal subjects. The periods of incubation in the experimental disease varied from 8 to 48 hours. We failed to obtain these results with the filtered secretions from cases of common colds 18 and 20 hours after the onset of symptoms and from a patient with the experimental disease 20 hours after the first symptoms. It would appear that the secretions are more active in the early hours of the affection. We also failed in the two instances in which colds were caused by exposure to the elements, or chilling of the body, and not by definite contact with other cases of common colds.

Intratracheal inoculations in rabbits with unfiltered and filtered nasopharyngeal washings obtained from patients with common colds induce no characteristic or distinctive effects on the tissues, from which no constant, pathogenic agent has, as yet, been recovered. In comparison, similar material from cases of epidemic influenza do, however, cause particular changes in the blood and lungs of these animals, cultures of the lungs often yielding *Bacterium pneumosintes*.¹⁻³ In view of these facts and since the clinical pictures exhibited by these diseases differ, the conclusion may be drawn that infectious common colds and epidemic influenza are separate and distinct diseases. On the other hand, the negative results obtained with materials derived from common colds and from parallel series of experiments with secretions

from supposedly healthy persons, serve as a control to the effects produced with the nasopharyngeal washings obtained from influenza patients.¹⁻³

Aerobic and anaerobic cultures of the filtered nasopharyngeal washings from 40 early cases of infectious common colds have thus far yielded no constant, pathogenic agent which can be regarded as the incitant of the disease. The filtered washings of nineteen cases were studied by the combined method of Smith-Noguchi fluid medium and anaerobic blood agar plates. In these instances representatives of the three groups of anaerobic filter-passing, Gram-negative bacteria, described by Olitsky and Gates² were cultured from twelve patients. The irregularity of their occurrence not only in common colds but in influenza and supposedly normal persons and their lack of pathogenicity for rabbits and man indicate that these bacteria are not peculiar to common colds. This method has opened to view a number of hitherto undescribed microorganisms which can be found in different respiratory affections and in health. Furthermore, by morphological, cultural, and serological means, the separation into distinct species of each of these groups of bacteria has again been demonstrated. It is noteworthy that *Bacterium pneumosintes* was not found in any of the cultures from the 40 patients.

Special attention was given to the detection of elements similar to Foster's globoid bodies in the cultures derived from common colds and from the experimental disease in man, and from the lungs of inoculated rabbits. We have not been able to determine the presence of these bodies, although the precipitate which forms in fresh rabbit kidney tissue-ascitic fluid medium was illusory in such relation since it was a common experience to find this precipitate simulating the globoid bodies of poliomyelitis. Still more disturbing is the fact, that these particles could be carried over from subplant to subplant and even showed pseudo colony formation in the Noguchi semisolid medium in tubes.⁸ But when the particles were put to rigorous test for a living and multiplying organism, the tests failed to reveal multiplication.

The increasing importance of the tissue-ascitic fluid medium in bacteriological technique warrants a detailed description of the requirements necessary for the determination of the living nature of formed

elements in cultures in this medium. (a) No one method of staining can be relied upon, for stain precipitate in itself adds to the confusion. A specimen for examination should be stained separately by Gram's and Giemsa's methods and with another nuclear dye, such as polychrome methylene blue. As a rule, microorganisms will reveal their morphological characters in more than one stain, whereas precipitate may be found in only one and not in the others. The experienced eye will discern the precipitated particles in selected parts of the stained preparation where they often occur in enormous numbers, clumped into irregular masses of varying forms from the periphery of which there is a gradual fading out to finer, more uniform structures. (b) In addition, suspected growths should be tested in the dialysate medium of Gates¹⁶ since by this method the precipitated material of the medium is kept from admixing with the growth, and a clear view of any microorganisms, if present, is obtained. (c) Another requirement is colony formation of the suspected culture. This is an absolute essential and can be effected by planting the material to be tested on solid plate media, incubated aerobically, and anaerobically in Brown's⁹ jar. Semisolid medium in long tubes should also be employed but care is needed to avoid mistaking small particles of precipitate for actual colonies of bacteria. To make certain of growth of microorganisms in semisolid medium, however, subplanting to a precipitate-free, dialysate medium is required.

By following this method minute microorganisms which are obscured, or simulated, by precipitate in the Smith-Noguchi medium, can be identified.

CONCLUSIONS.

The transmission of a clinical condition similar to typical, infectious common cold from man to man with the filtered nasopharyngeal washings of early cases of the disease indicates that the incitant is filterable, thus confirming the earlier observations of Kruse and Foster.

¹⁶ Gates, F. L., *J. Exp. Med.*, 1922, xxxv, 635.

Experiments on rabbits with these secretions and cultivation tests show that the materials derived from patients with common colds are distinct in effects from those of epidemic influenza.

Cultivations of the nasopharyngeal washings from 40 cases, and from the lung tissue of inoculated rabbits, have failed to reveal any constant, pathogenic agent, or incitant. Although a careful search was made for the "globoid bodies" of Foster in these materials, we have been unable to find them. None of these cultures, furthermore, yielded *Bacterium pneumosintes*.