THE ETIOLOGY OF ACUTE UPPER RESPIRATORY INFECTION (COMMON COLD)*

By PERRIN H. LONG, M.D., JAMES A. DOULL, M.D., JANET M. BOURN, PH.D., AND EMILY McCOMB, PH.D.

(From the Department of Medicine and the Clinical Laboratory of the John J. Abel Fund for Research on the Common Cold, The Johns Hopkins University, Baltimore)

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In 1914 Kruse (1) reported that he had produced colds in human volunteers by intranasal inoculations with Berkefeld filtrates of the nasal secretions from individuals ill with natural colds. The nasal secretions were diluted in fifteen volumes of physiological salt solution and filtered through a small Berkefeld candle. A few drops of this filtrate were instilled into the nostrils of twelve volunteers. After an incubation period of from 1 to 3 days, four of the twelve developed colds. Later in the sa ıe year the experiment was repeated and of the thirty-six individuals who were inoculated, fifteen developed colds with an incubation period varying from 1 to 4 days. In both instances it was not possible to demonstrate bacteria in the filtrates by means of aerobic or anaerobic cultures methods. These results led Kruse to conclude that the infectious agent of colds was a filtrable virus and to this virus he gave the name—*A phanozoum coryzae.*

A short time after Kruse's note appeared, Foster (2), in a careful study confirmed and extended Kruse's observation. Foster obtained the nasal secretions from early acute colds and after diluting and shaking them in salt solution, the mixture was filtered through a tested Berkefeld N candle. Seven of the ten healthy young men inoculated intranasally with a few drops of the filtrate developed typical colds within 48 hours. Human blood-agar plates prepared from the filtrates and incubated at 37°C., under both aerobic and anaerobic conditions,

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remained sterile at the end of 7 days. However, in anaerobic cultures prepared according to the Smith-Noguchi technique, minute spherical bodies were observed which were considered to be organisms and which in the first subculture generation proved infective, producing colds in ten out of eleven inoculated volunteers. In conclusion, Foster stated that he believed that the infectious agent of the common cold was a filtrable virus and that by utilizing a specialized culture medium, he was able to cultivate from filtrates containing active virus, a peculiar minute filter-passing microorganism which had a direct causal relation to colds.

In 1917 Dold (3) reported that he had been able to transmit an upper respiratory infection to human volunteers by means of a bacteria-free Berkefeld filtrate of the nasal secretions obtained from an individual ill with a natural "cold." The nasal secretions were diluted in fifteen volumes of physiological saline and then filtered through a Berkefeld candle under negative pressure. In the first group of experiments seventeen volunteers were inoculated with the Berkefeld filtrate. Fifteen students who worked, ate and slept in the same rooms with the volunteers served as controls. Seven of the inoculated volunteers (41 per cent) developed typical "colds" within 72 hours. None of the controls developed the affection during the period of observation. In a second experiment forty patients on the surgical wards were inoculated with a Berkefeld filtrate of the diluted nasal secretions from an individual ill with a "cold." The results were essentially negative as only one developed an upper respiratory infection. The filtrates were cultured in serum agar, ascitic agar and in ascitic broth under both aerobic and anaerobic conditions. Special cultures were made in a modified Smith-Noguchi medium in which ascitic broth replaced the usual ascitic fluid. The cultural results were negative in all instances. Diluted material from a 48 hour Smith-Noguchi culture of the filtrate of nasal secretions obtained from a volunteer in the first group who developed a "cold" subsequent to inoculation, was used in the third experiment. Two of these volunteers inoculated with this material developed mild upper respiratory symptoms which endured 4 or 5 days. The culture from which this material was obtained, was considered to be free of bacteria. In this paper experimental observations are also reported to the effect that, irritating substances (insect

powder) can when inhaled, produce the symptoms and signs of an acute infectious "cold."

During the influenza epidemics of 1919 and 1920, Schmidt (4) inoculated one hundred and ninety-six persons with Berkefeld filtrates of nasal secretions from colds and eighty-four individuals with Berkefeld filtrates of the respiratory tract secretions from individuals all with "grippe." In the first group, twenty-one developed colds and three developed grippe; while in the second group, five developed grippe and four colds. Of forty-three controls who had been inoculated with sterile physiological salt solutions eight developed colds. No information is given in this report as to the exact size of the Berkefeld filter or to the period in the disease when the secretions were collected. Schmidt in concluding his report, expresses the belief that more human beings must be inoculated before the true nature of the infectious agent is determined.

Shortly after this communication appeared, Williams, Nevens and Gurley (5) reported that they had been unable to infect forty-five volunteers with Berkefeld N filtrates of the nasopharyngeal washings from seven early "cold" cases and three typical influenza cases.

During investigations concerning the etiology of influenza, Olitsky and McCartney (6) studied the filtered nasopharyngeal secretions of numerous individuals, ill with "colds." They were unable to produce any characteristic lesions in rabbits, by intratracheal inoculation with Berkefeld V or N filtrates of the nasopharyngeal washings from these subjects. However, they were able to cultivate several groups of minute anaerobic Gram-negative organisms from some of these filtrates. In their opinion, none of these minute organisms were constant enough to be considered the incitant of the affection. By using as the inoculum, filtered nasopharyngeal secretions from early cases of typical infections, they were able to transmit singly and in series upper respiratory infections to human volunteers, thus demonstrating that the incitant was a filtrable agent.

Robertson and Groves (7) collected the nasal secretions of persons ill with uncomplicated coryza from 6 to 144 hours after the onset of the infection. After diluting these secretions with sterile salt solution, the mixtures were filtered through Berkefeld candles. The filtrates were cultured upon aerobic and in anaerobic media and in every

instance the cultures remained sterile. Smears from the filtrates invariably showed coccoid bodies similar to those described by Foster. No significance was attached to these findings since the same bodies could be demonstrated in filtrates of the nasal washings from normal persons. "Inoculations with the filtrates were made into the nostrils of volunteers as soon after filtration as possible. In no case did this time exceed twenty-four hours after the collection of the secretions, which in the meantime were kept in the ice-box." One hundred human volunteers were inoculated with the various filtrates and five of them developed upper respiratory infections following the inoculations. Because of the small number of positive results, these observers considered them to be the result of causes entirely independent of the inoculations, and concluded that their experiments presented no convincing evidence indicative of a filtrable agent as being the exciting factor in acute coryza.

In an extensive study of acute upper respiratory infection, Dochez and his associates have investigated all of the possible etiological factors in this affection. Early in the course of their investigations (8) they demonstrated that the aerobic flora of the nasopharynx played little, if any, rôle in the production of infectious "colds." Later (9) in a study designed to ascertain the etiological significance of the anaerobic filter-passing flora of the nose and throat, similar conclusions were drawn in regards to anaerobic microorganisms. Recently, Dochez, Shibley and Mills (10) reported that in the course of their investigation on human upper respiratory infections, they were able to produce in apes, by means of inoculations with Berkefeld V filtrates of the nasopharyngeal washings from individuals ill with "colds," a condition which bore a striking resemblance to the human disease. In this communication they stated that as yet the active filtrable agent was unknown, but that from the filtrate in all positive experiments, a Gram-negative anaerobic bacillus of the type described by Olitsky and Gates, had been cultivated. In a further report (11) they stated that they had been unable to infect apes with the filtrates of nasopharyngeal washings from normal individuals, notwithstanding the fact that from 75 per cent of these filtrates, they cultivated Gram-negative anaerobes. These findings led them to believe that the type

of upper respiratory infection under consideration was caused by a filtrable virus.

In two recent reports Dochez and and his associates (12, 13) describe the results of their transmission experiments in apes and in human beings. Of twenty-eight ape experiments concerned directly with the testing of the hypothesis that colds may be caused by filtrable agents, washings from individuals with colds were used in twenty instances and normal washings in eight. Four animals in the first group were excluded for statistical purposes. Seven of sixteen animals contracted colds subsequent to inoculation with filtered nasal washings from individuals ill with natural colds. In the group of eight animals inoculated with filtrates of nasal washings from normals, none developed colds, although 75 per cent of these filtrates contained Gram-negative filter-passing microorganisms. Several miscellaneous experiments were performed including two successful ape to ape transmissions in which filtered ape washings were used and two unsuccessful experiments in in which an attempt was made to transfer colds by means of living cultures of the filter-passing anaerobes obtained from a filtrate which had been used in a successful transmission experiment. In the course of their experimental transmission tests in apes several positive results were obtained with filtrates in which the filter-passing anaerobes were absent, thus substantiating their view that the filter-passing anaerobes were not the primary etiological agents in the production of "colds."

The human transmission tests were carried out under a most rigorous system of quarantine in which every precaution was taken to protect the subjects from direct or indirect contact with natural colds. Nine completed experiments are reported in which transmission was attempted by means of filtered nasal washings and of these four were successful. As a result of their experimental observations these investigators conclude that "the contagious cold in human beings is caused by an invisible, uncultivable, filtrable agent which in all likelihood belongs to the group of so-called submicroscopic virus."

The observations to be recorded in this paper have already been briefly referred to in a short report (14). They were undertaken at a time when the most recent communications of Dochez and his associates were in the course of publication. As will be seen from the text, our observations confirm their experimental work and support the

conclusions of Dochez and his associates that the contagious "cold" is caused by a filtrable agent which probably belongs to the group of so called filtrable viruses.

Methods

Healthy, intelligent young women were selected as volunteers for the test. A careful history, in which special emphasis was placed upon previous upper respiratory infection, was taken in each instance. The nose and throat of each subject was examined* and roentgenograms of the sinuses and chest were taken before accepting the applicant. The first twelve tests were performed upon the volunteers who were kept in single rooms on the isolation ward. There were no other patients in this ward during the experimental periods. In the second group of eight tests, in order to avoid having subjects with upper respiratory infection on the same pavilion in which other subjects were passing through the required control period, the volunteers were placed in pairs in suites of rooms on the private wards and on the isolation ward. This plan ruled out the possibility of accidental infection through direct contact, and allowed us to utilize the patients on the private wards as a control group. It also permitted test transfers to widely separated groups of individuals.

The volunteers were kept in strict quarantine. The nursing care was performed by graduate nurses. Only one observer (P. H. L.) came into contact with the subjects until symptoms had developed. Hands were scrubbed and sterile masks and gowns donned before coming into contact with the individual volunteer. All eating utensils were sterilized. The nurses and the observer were examined daily for upper respiratory infection.

Each subject had a thorough physical examination upon entry. White blood cell counts were taken daily and the urine was examined frequently. Observations of the temperature, pulse and respirations were made at 4 hour intervals during the control and incubation periods, and at two hour intervals after the onset of symptoms.

Daily aerobic cultures of the tonsils and posterior pharyngeal wall were examined for the presence of *H. influenzae* and *Streptococcus haemolyticus*, type Beta. In studying *H. influenzae* the sodium oleate hemoglobin agar plates (pH 7.4) described by Avery (15) were employed. The technique of isolation and identification of *H. influenzae* on these plates has already been described by Bourn (16) in her studies of these organisms.

The examinations for the presence of *Streptococcus haemolyticus*, type Beta, were made by placing the nasopharyngeal swab in beef infusion broth (pH 7.4–7.6) and allowing it to incubate for at least 2 hours at room temperature. Dilutions of the two-hour cultures were inoculated into liquid 5 per cent whole rabbits' blood

* The nose and throat examinations were performed by either Dr. J. J. Chisolm or Dr. S. W. Egerton of the Department of Otolaryngology.

agar, and plates were poured. After 24 hours incubation at 37°C. these plates were examined for the presence of hemolytic colonies and identification was completed by fishing individual colonies for smear preparations.

During the control period and after inoculations, nasopharyngeal swabs were examined for the presence of *D. pneumoniae*. These swabs were suspended in whole rabbits' blood broth and were incubated at room temperature for several hours. Then 1 cc. of the blood broth suspension was injected into the peritoneum of a white mouse and whole rabbits' blood agar plates were streaked with material from the suspension. The usual white mouse technique for the determination of pneumococci was followed and pneumococcus-like organisms were tested for bile solubility and typed serologically by means of Groups I, II and III specific anti-pneumococcus serum. The anaerobic filter-passing flora of the nose was studied at entry and further studies of these organisms were made after inoculation. The material for the anaerobic studies and for inoculation purposes was obtained by running 10 to 15 cc. of warm yeast infusion broth (pH 7.6) through the nasal passages and back into the nasopharynx. The washings were collected in a sterile bottle, shaken vigorously with glass beads and filtered either through tested Berkefeld V or W candles or through a small Seitz filter (Uhlenhuth model) (17). Cultivation of the filtrates was carried out in Smith-Noguchi medium (Olitsky and Gates modification), in yeast infusion broth (pH 7.6), and upon 5 per cent rabbits' blood dextrose yeast infusion agar plates. All cultures were incubated 10 to 14 days before being discarded.

The subjects with natural colds, from whom the inoculum was obtained, were carefully selected. The type of infection was investigated in an effort to rule out hay fever and acute sinus infections. The inoculum was obtained in each instance during the early hours of the affection. Regional aerobic cultures were planted and the nasopharyngeal washings were quickly passed through the desired filters. Not more than ½ hour elapsed from the time of the washing until the subjects were inoculated.

The volunteers were observed during a control period of from 2 to 6 days (generally 5 days) prior to inoculation. During this time they received from two to five nasal instillations of filtered yeast infusion broth which were given in the same manner as the inoculations and were designed as a check upon the psychological reactions of the subjects and also to prevent them from knowing the time of inoculation. The technique of inoculation was as follows: the volunteer was placed in a supine position with the head tipped back and from 0.5 cc. to 1.0 cc. of the filtrate was introduced into each nostril. Then the mucous membranes of the anterior nasal passages and the posterior pharyngeal wall were lightly rubbed with swabs soaked in the filtrate. Each individual received from one to three inoculations within a period of 24 hours.

As soon as a volunteer complained of symptoms, careful examinations of the nose and throat were made by several observers. These examinations were repeated at frequent intervals during the infection. Detailed records were kept of all symptomatic and objective findings. The presence of objective changes in the naso-

pharynx and nasal passages was considered necessary for the diagnosis of "a cold" in the inoculated individual.

Transmission Tests in Man

Protocols of Individuals Ill with Natural "Colds."—

Patient A.—No history of exposure. 6/11/30—Upon awakening, her head ached and her throat was sore. A thin watery nasal discharge and intermittent nasal obstruction were present. The nose and throat examination was essentially normal except for a small amount of nasal discharge. 6/12/30—Quite uncomfortable. Marked constitutional symptoms were present, and nasal discharge and obstruction more marked. The cold endured 5 days. No aerobic cultures were made. Anaerobic cultures of the Berkefeld V filtrate showed Group III A and a diphtheroid. Cultures of the Seitz filtrates were negative.

Patient B.—6/12/30—At 4 a.m., developed sneezing, irritation of the throat, and a profuse watery nasal discharge. Soon this became more profuse and nasal obstruction and profuse watery discharge. Examination: Skin of anterior nares was reddened and appeared to be irritated by the nasal discharge. Mucous membranes of the turbinates and septum were very red. Moderate amount of thin watery discharge seen in both nostrils. Tonsils small, the left tonsil inflamed; the posterior pharyngeal wall hyperemic. Aerobic cultures negative for *Streptococcus haemolyticus*, type Beta, and *H. influenzae*. Anaerobic cultures of the Berkefeld V filtrates showed Group IV, Group III A and an unidentified rod. Cultures of the Seitz filtrate were negative.

Patient C.—No known exposure. 6/11/30—Fatigue, drowsiness and smarting of the eyes. 6/12/30—On awakening, felt very tired; throat was sore, back ached and a thin watery nasal discharge with nasal obstruction was present. Examination showed slight swelling and hyperemia of the inferior turbinates on both sides. A small amount of mucoid nasal discharge present. The tonsils hyperemic and injected. Aerobic culture showed no hemolytic streptococci, type Beta, or *H. influenzae*. Anaerobic cultures and cultures of the Seitz filtrate negative. This infection endured for 5 days.

Patient D.—6/17/30—During the afternoon of 6/13/30 was exposed to an early cold. During the night of 6/15/30 developed sneezing and a sense of dryness and fullness in throat. On the following day throat was quite sore and intermittent nasal obstruction and a scant thin watery nasal discharge appeared; generally uncomfortable. Symptoms increased in intensity and by the next day he was in addition suffering from headache and slight malaise. Examination: Temperature 99°F. (by mouth). Mucous membranes of nose slightly injected. Good breathing space present on the right; on the left the inferior turbinates much enlarged. Practically no discharge was present on either side. Tonsils small; the mucous membranes of the posterior pharyngeal wall bright red. No evidence of sinus infection. The duration of affection was 7 days. Aerobic cultures negative for *Streptococcus haemolyticus*, type Beta, and *H. influenzae*. Anaerobic cultures of the Berkefeld

filtrates showed Groups IX, III A, and IV. Cultures of the Seitz filtrates remained sterile.

Patient E.—6/24/30—No history of previous exposure. During the evening of 6/22/30 noticed dryness and fullness of the throat which by the next morning had developed into mild sore throat, accompanied by sensations of warmth and cold, generalized aching and nasal discharge and obstruction. On the next day the same signs and symptoms persisted, with, in addition, hoarseness and cough. Examination: Temperature 99.2 (by mouth). Fair breathing space on both sides. The nasal mucous membranes slightly swollen and hyperemic. A small amount of thin watery discharge present. Tonsils very small and not inflamed. The posterior pharyngeal wall was injected and a slight amount of mucoid posterior nasal discharge was seen. The glands at the angles of the jaw were enlarged and tender. Aerobic cultures were negative for *Streptococcus haemolyticus*, type Beta, and *H. influenzae*. Anaerobic cultures of the Berkefeld V filtrate showed two unidentified organisms. Cultures of the Seitz filtrates remained sterile.

Patient F.—6/25/30—No known exposure. During the afternoon of 6/24/30 noticed a sensation of fullness, dryness and of irritation of the throat with chilliness, aching of the eyes, slight nasal discharge and slight nasal obstruction. After a restless night, throat was sore, eyes ached, nasal passages were irritated, with marked increase in the amount of nasal discharge and obstruction. There was slight malaise, and by afternoon hoarseness. Temperature 99.4°F. (by mouth). Good breathing space in both nostrils. Moderate hyperemia and slight swelling of the mucous membrane, with a small amount of watery discharge, found in the nasal passages. The pharynx and nasopharynx moderately hyperemic. Aerobic cultures negative for hemolytic streptococci, type Beta, and *H. influenzae*. Anaerobic cultures of the Berkefeld V filtrate showed Groups IV and III A. Cultures of the Seitz filtrates were negative.

Patient G.—6/25/30—No known exposure. During the morning of 6/24/30 developed a sensation of dryness, fullness and irritation of the throat. In a short time thin nasal discharge, nasal obstruction and lacrimation appeared. On the following day, an increase in the intensity of signs and symptoms plus a feeling of moderate malaise. Examination: Temperature 99.4°F. (by mouth). Good breathing space on the right. Turbinates and mucous membranes on right seemed normal. Poor breathing space on the left side due to quite marked deflection of septum and to hyperemia and swelling of the mucous membranes. Small amount of mucoid nasal discharge. The pharynx was moderately injected. Aerobic cultures negative for *Streptococcus haemolyticus*, type Beta, and *H. influenzae*. Anaerobic cultures of the Berkefeld V filtrate showed Group IV. Seitz filtrate yielded sterile cultures.

Patient H.—6/26/30—History of exposure to a draught during 6/25/30. Early in the morning of 6/26/30 developed a sensation of fullness, dryness and irritation in the throat, slight nasal discharge and intermittent nasal obstruction. On the following day these had increased in intensity and lacrimation, hoarseness

and cough had made their appearance, with a slight frontal headache. Examination: Temperature 98.6°F. (by mouth). Good breathing space on both sides. No definite changes in mucous membranes of nasal passages. A small amount of watery discharge on floor of each nostril. Five hemorrhagic spots on buccal mucous membranes. The pharynx slightly injected. No aerobic cultures were made.

Anaerobic cultures of the Berkefeld V filtrate showed Group XVII organisms. Culture of the Seitz filtrate negative.

Patient I.—No history of exposure 7/12/30. While in a motor during the evening noticed a slight watery anterior nasal discharge. Did not sleep well and on the next day developed a frontal headache, sneezing, anterior and posterior nasal discharge, intermittent nasal obstruction and a sore throat. On 7/14/30 these had increased in intensity and hoarseness, a cough, and a small amount of mucopurulent sputum were present. Examination: Temperature 99.4°F. (by mouth). The mucous membrane of the turbinates swollen and injected. Poor breathing space on both sides. Very little nasal discharge seen. The entire pharynx moderately hyperemic. No aerobic cultures were taken. Anaerobic cultures of Berkefeld V filtrate and cultures of Seitz filtrate negative.

Protocols of Transmission Tests.—The material for the inoculation of volunteers was procured from the patients whose clinical histories have just been given.

Volunteer I.—Age 20. Influenza in March, 1929. One "cold" during the past 2 years. Physical and nose and throat examinations normal. Five-day control period. Inoculated on 6/11/30 with Berkefeld V filtrate from Subject A and on 6/13/30 with a mixed Berkefeld V filtrate from Subjects B and C. No symptoms or signs of an upper respiratory infection developed; discharged on 6/16/30. On the 1st day of the control period Type IV pneumococci in cultures from the nasopharynx. Subsequent daily cultures negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae*. Anaerobic cultures negative. White blood cell counts and the urine revealed normal findings at all times.

Volunteer II.—Age 22 years. Influenza in 1918. One cold during the past 2 years. Physical and nose and throat examinations normal except for moderately hypertrophied tonsils and adenoids. Five-day control period. Inoculated on 6/11/30 with a Seitz filtrate of nasopharyngeal washings from Subject A and 6/11/30 with the mixed similar filtrates from Subjects B and C. 6/13/30—Slight fullness and dryness in throat and a slight anterior nasal discharge, which disappeared within a few hours; discharged on 6/17/30. During the first 4 days of control period and on the 3rd and 5th days after inoculation, showed non-hemolytic *H. influenzae* in cultures from nasopharynx. Hemolytic *H. influenzae* present on the 3rd day after inoculation. No hemolytic streptococci, type Beta, or *D. pneumoniae* found. Anaerobic cultures taken before inoculation showed no growth, while from similar cultures taken after inoculation Group III A and XVII organisms were isolated. White blood cell counts and the urine were normal at all times.

Volunteer III.—Age 26 years. Bronchitis (?) 1928. Tonsilitis January, 1930. One cold during the past 2 years. Physical and nose and throat examinations normal except for slight irregularities in the nasal septum. Five-day control period during which an herpetic vesicle appeared on the lip and then retrogressed. Inoculated on 6/12/30 with a Berkefeld V filtrate of nasopharyngeal washings from Subject B. No symptoms of signs of an upper respiratory infection developed; on 6/17/30 was discharged. Aerobic cultures from the nasopharynx negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. The anaerobic culture showed no growth. White blood cell counts and the urine showed no abnormalities.

Volunteer IV.—Age 20 years. Pneumonia in 1916. Six colds during the past two years. Physical examination normal. Nose and throat examination showed questionable chronic tonsillitis. Five-day control period. On 6/12/30 inoculated with the Seitz filtrate of nasopharyngeal washings from Patient B. 36 hours later complained of sense of dryness, fullness and irritation in throat. Nose and throat at this time showed no changes and by the next day the symptoms had disappeared. Discharged on 6/17/30. Atypical hemolytic streptococci, type Beta, isolated in every culture from the nasopharynx. Non-hemolytic *H. influenzae* in the 5th day of the control period and on the 3rd day after inoculation. All anaerobic cultures remained sterile. White blood cell counts and the urine remained normal throughout.

Volunteer V.—Age 24 years. Two colds during the past 2 years. Physical and nose and throat examinations normal. Three-day control period. Inoculated on 6/17/30 with Berkefeld V filtrate of nasopharyngeal washings from Patient D. 54 hours after this, dryness, fullness and irritation in throat. Examination of nose and throat showed no significant changes. The symptoms rapidly disappeared; discharged on 7/21/30. The aerobic cultures from the nasopharynx negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures showed Group IV organisms both before and after inoculation. The white blood cell counts and the urine were normal throughout.

Volunteer VI.—Age 21 years. Tonsillitis 1927. Acute frontal sinusitis 1927. Two colds during the past 2 years. Physical examination normal. Nose and throat examination showed a deflected septum and a mild chronic infection in the right tonsil. Three-day control period. The subject was inoculated on 6/17/30 with the Seitz filtrate of nasopharyngeal washings from Patient D. On 6/19/30, 50 hours after she complained of a sensation of dryness, fullness and of irritation in throat; nasal passages were obstructed. Slept with difficulty. 6/20/30—Developed a slight watery nasal discharge. Towards evening this became more marked and a high-grade of nasal obstruction was present. 6/21/30—Marked nasal obstruction and a moderate amount of mucoid nasal discharge, with slight headache and malaise. Temperature 99.2°F. (by mouth). Examination showed moderate hyperemia and swelling of nasal mucous membranes and a moderate

amount of mucopurulent discharge on the floor of each nostril. The entire pharynx was slightly injected. 6/22/30—The nasal discharge and obstruction persisted although the subject felt much better. Temperature 99.2°F. (by mouth). 6/23/30—Marked decrease in the discharge and obstruction. 6/24/30—Very little nasal discharge. Crusting in both nostrils. Slight nasal obstruction. Volunteer was discharged on this day. Aerobic cultures from the nasopharynx were negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures showed Group IV organisms both before and after inoculation. The white blood cell counts and urine were normal throughout.

Volunteer VII.—Age 21 years. Tonsillectomy and adenoidectomy in 1918. Two colds during the past 2 years. Physical and nose and throat examinations normal, except for a deflected septum. Six-day control period. Inoculated on 6/24/30 with a Berkefeld V filtrate of nasopharyngeal washings from Patient E. On 6/26/30, 50 hours after this inoculation, a sensation of fullness and irritation in throat, sneezing and of a thin watery nasal discharge. Nose and throat examination: slight diffuse hyperemia of nasal and pharyngeal mucous membranes and a small amount of thin watery nasal discharge. 6/27/30—Nasal discharge while profuse was quite intermittent as was also nasal obstruction. Moderate hoarseness and coughing. Nose and throat examination showed only slight changes from normal. Volunteer forced to leave the hospital on 6/27/30 and was followed by telephonic reports. On 6/28/30 nasal obstruction and discharge present. Temperature 99.2°F. (by mouth). Hoarseness had decreased in severity. 6/29/30—Hoarseness and coughing persisted. Intermittent nasal discharge. 6/30/30—Intermittent nasal discharge and obstruction, with crusting. 7/1/30—Nasal discharge was scant and crusting had increased. 7/2/30—Nose practically normal. Non-hemolytic *H. influenzae* isolated in cultures from the nasopharynx on the 1st and 5th days of the control period. *D. pneumoniae*, hemolytic streptococci, type Beta, and hemolytic *H. influenzae* were not isolated in any cultures from the nasopharynx. Anaerobic cultures taken in the control period were negative while those obtained on the 3rd day after inoculation showed Group III A organisms. White blood cell counts and the urine were normal throughout.

Volunteer VIII.—Age 22 years. Tonsillectomy and adenoidectomy in 1925. One cold during the past 2 years. Physical and nose and throat examinations normal. Six-day control period. Inoculated on 6/24/30 with the Seitz filtrate of nasopharyngeal washings from Patient E. 24 hours after this, a sensation of fullness, dryness and of irritation in the throat, slight nasal obstruction and a slight amount of thin watery nasal discharge; felt quite listless and drowsy. Did not sleep well. 6/26/30—Moderate mucoid anterior and posterior nasal discharge, nasal obstruction, headache, mild malaise, soreness in the nasal passages and throat, and a temperature of 99.4°F. (by mouth). Nose and throat examination: Good breathing space on both sides. Inferior turbinate on right swollen and hyperemic. A small amount of clear mucoid discharge seen on the floor of both nostrils. In

the nasopharynx the adenoid tags were injected and swollen. Posterior wall of the pharynx completely covered with clear mucoid discharge. 6/27/30—Nasal discharge more purulent in character and nasal obstruction more marked. Constitutional symptoms had disappeared but hoarseness had appeared. Nose and throat examination was unchanged. 6/28/30—Nasal discharge greatly lessened in amount and quite thick; nasal obstruction intermittent. A moderate productive cough with small amounts of tenacious mucoid sputum developed and hoarseness was more marked. Examination: Nose—no discharge or swelling seen. The mucous membrane of the nasopharynx was injected. The pharynx was moderately injected with some mucoid discharge over the entire posterior wall. The mucous membrane of the larynx slightly injected. Volunteer was forced to leave on this date but from subsequent reports was completely well within 5 days. Aerobic cultures from nasopharynx negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures showed Group IV organisms both before and after inoculation. The white blood cell counts and the urine were normal throughout.

Volunteer IX.—Age 22 years. Influenza in 1918. Tonsillectomy and adenoidectomy in 1923. Three colds during the past 2 years. Physical examination showing nothing abnormal. Nose and throat examination was essentially normal. Three-day control period. Inoculated on 6/24/30 with a Seitz filtrate of nasopharyngeal washings from Patient E, on 6/25/30 with a mixed Seitz filtrate from Patients F and G and on 6/26/30 with a Seitz filtrate from Patient H. No definite symptoms or signs resulted from these inoculations; discharged on 7/1/30. Nonhemolytic *H. influenzae* isolated in cultures from nasopharynx on 1st day of control period and on 2nd, 3rd and 4th days after inoculation. *D. pneumoniae, Streptococcus haemolyticus*, type Beta, and hemolytic *H. influenzae* not isolated at any time. Anaerobic cultures showed Group IV organisms both before and after inoculation. The white blood cell counts and the urine were normal throughout.

Volunteer X.—Age 20 years. Nasal polyps removed in 1916. Tonsillectomy and adenoidectomy in 1929. Two colds during the past 2 years. Physical examination normal except for marked deafness in left ear. Nose and throat examination normal. Three-day control period. Inoculated on 6/24/30 with a Berkefeld V filtrate of nasopharyngeal washings of Patient E, on 6/25/30 with a mixed Berkefeld V filtrate from Patients F and G, and on 6/26/30 with a Berkefeld V filtrate from Patient H. 43 hours after the first inoculation, nasal obstruction, watery nasal discharge, sneezing and a slight feeling of malaise. Did not sleep very well because of nasal obstruction and on 6/28/30 had a profuse bilateral nasal discharge, nasal obstruction, hoarseness, a sore throat, sneezing and tender glands at angle of jaw on left side. During the day used seventy-four gauze handkerchiefs. Examination showed that the left inferior turbinate was greatly swollen causing marked obstruction on the left side. The mucous membranes of the nasal passages were injected and sticky mucoid nasal discharge present in both nostrils. The pharynx

was normal. The glands at the angles of the jaw were tender. Temperature rose to 99.6°F. (by mouth). 6/29/30—The nasal discharge was mucopurulent and nasal obstruction less marked. No malaise. A slight conjunctivitis; eyes tired easily. The glands in the neck still tender. Forty gauze handkerchiefs were used during the day. 6/30/30—The nasal discharge much thicker and decreased in amount. Nasal obstruction intermittent. Crusting had commenced. No malaise. 7/1/30—Moderate anterior and posterior nasal discharge. Slight obstruction, and more marked crusting. Nose and throat examination: The remains of a rapidly clearing acute nasopharyngeal process. At the patient's request, discharged on this day. Non-hemolytic *H. influenzae* isolated in cultures from nasopharynx on the 3rd day of the control period and upon the 4th day after inoculation. *D. pneumoniae*, hemolytic *H. influenzae* and hemolytic streptococci, type Beta, not isolated. Anaerobic cultures showed Group IV and XIV organisms during the control period and Group IV and III A after inoculation. Cultures of the Seitz filtrates negative. The white blood cell counts and the urine were normal throughout.

Volunteer XI.—Age 20 years. Tonsillectomy and adenoidectomy in 1916. Two colds during the past 2 years. Physical examination was normal except for a small colloid goiter and a marked respiratory arhythmia. Nose and throat examination showed both tonsil and adenoid remnants. Two-day control period. Inoculated on 6/28/30 and again on 6/29/30 with Seitz filtrates of nasopharyngeal washings from Volunteer X. 70 hours after this, marked lassitude, headache and backache; remained in bed. Temperature 100°F. (by mouth). Nose and throat examination showed poor breathing space on the left. The mucous membranes of the left lower inferior turbinate injected and swollen and a small amount of mucoid discharge on this side. 7/2/30—Moderate nasal discharge and obstruction and slight sore throat. Nose and throat examination showed swelling and injection of the mucous membranes in the nasal passages and a small amount of thick mucoid discharge. The pharynx was injected. Two hemorrhagic spots seen on the buccal mucous membrane of right cheek. 7/3/30—The nasal symptoms and signs remained unchanged. During the day suffered from generalized malaise, headache, backache, and a sharp attack of diarrhoea, accompanied by some abdominal discomfort. 7/24/30—The nasal findings were unchanged. Marked lassitude and moderate backache. No diarrhoea. 7/5/30—Moderate malaise and moderate diarrhoea during the morning. Temperature 100.4°F. (rectal). Much less nasal discharge and nasal obstruction present. Examination of the nose and throat showed that the rhinitis was clearing. Discharged on 7/6/30. The urine was normal throughout. No abnormal bacteriological findings were noted in the stool examination made during the periods of diarrhoea. The aerobic cultures from the nasopharynx were negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic culture showed Group IV and XVII organisms during the control period and Group IV organisms after inoculation. Cultures of the Seitz filtrates were negative.

6/27/30	W.B.C.—	7120	
6/28/30	"	7200	Inoculated with Seitz filtrate
6/29/30	"	6080	R.B.C. 4,320,000. Hgbn. 100 per cent
6/30/30	"	5360	
7/ 1/30	W.B.C.—	5280	Nasal signs
7/ 2/30	"	6320	
7/ 3/30	"	4560	Diarrhoea
7/ 4/30	"	4200	
7/ 5/30	"	5320	Diarrhoea
7/ 6/30	"	6200	

Volunteer XII.—Age 20 years. Two colds during the past 2 years. Physical examination showed nothing abnormal. Nose and throat examination showed a deflected septum and some enlargement of the posterior half of the right inferior turbinate. Four-day control period. Inoculated on 7/2/30 with a Seitz filtrate of nasopharyngeal washings from Volunteer XI. 41 hours after this was very tired, and nasal obstruction with a slight watery nasal discharge developed. Towards evening, became moderately hoarse. 7/5/30—A moderate nasal discharge and nasal obstruction. No constitutional symptoms with the exception of a temperature of 100°F. (rectal). Examination showed swelling and hyperemia of both inferior turbinates together with a small amount of mucoid discharge on the left. The vessels of the soft palate and pharynx were engorged. Two hemorrhagic spots present in the buccal mucous membrane on the left side. 7/6/30—Nasal discharge thicker, the nasal obstruction less marked, and crusting had begun. The nasal passages felt very raw, and examination showed increased injection of the nasal mucous membrane but less swelling. A small amount of thick, sticky discharge seen on the floor of the nostrils. The entire pharynx slightly hyperemic. 7/7/30 —The nasal discharge had markedly decreased as had also the nasal obstruction. Marked crusting. 7/8/30—Practically no nasal discharge or obstruction. Examination revealed normal nasal passages. The pharynx was moderately hyperemic. Volunteer was discharged on this day. The aerobic cultures from the nasopharynx were negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures showed Groups XVII and IV during the control period and Groups XVII and III A after inoculation. The white blood cell counts and the urine were normal throughout.

Volunteer XIII.—Age 28 years. Influenza in 1918. One cold during the past 2 years. Physical and nose and throat examinations normal except for two hemorrhagic spots on the buccal mucous membrane of the left cheek. Five-day control period. Inoculated twice on 7/14/30 and once on 7/15/30 with Seitz filtrates of the nasal washings from Patient I. No symptoms or signs of an upper respiratory infection developed and on 7/17/30 the volunteer was discharged. The aerobic cultures from the nasopharynx negative for hemolytic streptococci, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. A diphtheroid was isolated from the anaerobic cultures both before and after inoculation. The white blood cell counts and the urine were normal throughout.

Volunteer XIV.—Age 18 years. Influenza in 1928. Tonsillectomy and adenoidectomy in 1921. Two colds during the past 2 years. Physical examination showed nothing abnormal. Nose and throat examination showed a deflection of the septum and slight hyperemia of the inferior turbinates; one hemorrhagic spot on left buccal mucous membrane. Five-day control period. Inoculated twice on 7/14/30 and once on 7/15/30 with Seitz filtrates of nasopharyngeal washings from Patient I. 24 hours after the first inoculation, hoarseness, slight soreness in the region of the uvula, a thin watery nasal discharge and nasal obstruction. 7/16/30—Signs and symptoms were increased in intensity. Examination: The right inferior turbinate was enlarged and congested, the right middle turbinate pale. The mucous membranes in the left nostril hyperemic. A small amount of mucoid discharge present. The throat was moderately injected. The glands at the angles of the jaw were slightly enlarged and tender. 7/17/30—During the day abundant anterior and posterior nasal discharge and constant nasal obstruction. Examination: The mucous membranes of the nasal passages were hyperemic, and the right turbinate enlarged. More discharge than on the previous day. The pharynx was hyperemic. 7/18/30—Severe frontal headache. Temperature was 100.8°F. (rectal). Nasal symptoms and signs remained unchanged. 7/19/30—No headache. No nasal discharge and obstruction had decreased. Temperature 100°F. (rectal). Examinations revealed no changes suggestive of sinus involvement. 7/20/30—Practically no nasal discharge. 7/21/30—No nasal discharge. Nose and throat examination was similar to that of entry. Hemolytic streptococci, type Beta, were isolated in all cultures from the nasopharynx. Aerobic cultures from the nasopharynx were negative for *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures showed Group IV organisms both before and after inoculation. Cultures of the Seitz filtrates negative. A slight rise in the white blood cells upon the 3rd, 4th and 5th days after inoculation. The urine was normal throughout.

Volunteer XV.—Age 21 years. Pneumonia in infancy. Tonsillectomy in 1913. Influenza in 1918. Three colds during the past 2 years. Physical and nose and throat examinations normal. Inoculated twice on 7/16/30 and once on 7/17/30 with Seitz filtrates of nasopharyngeal washings from Volunteer XIV. On 7/18/30 4 hours after the first inoculation, a sensation of dryness and irritation in throat, thin watery nasal discharge, nasal obstruction, frontal headache and slight malaise. Twenty-five gauze handkerchiefs were used during the day. Examination revealed no changes in the nose. The lymphoid follicles on the posterior pharyngeal wall were larger and redder than previously. Two hemorrhagic spots on the left buccal mucous membranes. A small tender gland at the angle of the jaw on the left side. 7/19/30—The nasal discharge profuse and nasal obstruction more marked. Throat sore, hoarseness and a slight cough, with loss of taste. Fifty gauze handkerchiefs were used during the day. Examination showed definite congestion of mucous membranes in the nasal passages. A large amount of thin mucopurulent discharge in the left nostril. The lymphoid follicles on the posterior pharyngeal

wall slightly more enlarged and inflamed. 7/20/30—The nasal discharge was thick and was decreased in amount. Nasal obstruction intermittent. Hoarseness had about disappeared and coughing was infrequent. 7/22/30—Very little nasal discharge; practically no obstruction. Cough much improved. 7/23/30—No clinical remains of the infection. The nose and throat examination practically normal. *D. pneumoniae*, Type IV, in all aerobic cultures from the nasopharynx and non-hemolytic *H. influenzae* on the 4th day of the control period and upon the 2nd, 5th and 6th days after inoculation. No hemolytic streptococci, type Beta. Anaerobic cultures negative during the control period but showed Group III A organisms after inoculation. Cultures of the Seitz filtrates negative. White blood cell count slightly elevated on the 2nd, 3rd, 4th and 5th days after inoculation. The urine was normal throughout.

Volunteer XVI.—Age 20 years. Influenza in 1918. Tonsillectomy and adenoidectomy in 1920. Two colds during the past 2 years. Physical and nose and throat examinations normal. Five-day control period. Inoculated twice on 7/16/30 and once on 7/17/30 with Seitz filtrates of nasopharyngeal washings from Volunteer XIV. During the evening of 7/17/30 temperature was 100.8°F. (rectal), but there were no symptoms or signs of upper respiratory disease. 7/18/30—At noon, complained of nausea, abdominal discomfort, headache, backache, aching in muscles and was extremely listless; towards evening diarrhoea developed. 7/19/30—Diarrhoea was checked but the marked listlessness, headache, and backache remained. Examination of the nose and throat normal. 7/20/30—No diarrhoea. Patient still very listless. Temperature 100°F. (rectal). 7/21/30—Felt very well. 7/23/30—Volunteer was discharged. The urine was normal throughout. The aerobic cultures from the nasopharynx were negative for *Streptococcus haemolyticus*, type Beta, *H. influenzae* and *D. pneumoniae* throughout the experiment. Anaerobic cultures were negative both before and after inoculation. White blood cell counts were within normal limits.

Volunteer XVII.—Age 18 years. Tonsillectomy and adenoidectomy in 1920. Grippe in 1927 and 1929. Two colds in the past 2 years. Said that all of her colds were "grippy" in nature. Physical and nose and throat examinations normal except for one hemorrhagic spot on each cheek. Five-day control period. Inoculated twice on 7/19/30 and once on 7/20/30 with Seitz filtrates of the nasopharyngeal washings from Volunteer XV. 20 hours after the first inoculation, chilliness, weakness, irritability, aching in the eyes and neck, lacrimation, sneezing, a thin watery nasal discharge, partial nasal obstruction, loss of appetite and taste and soreness and rawness in the nasopharynx and nasal passages. Slept very poorly. 7/21/30—Patient looked ill, and complained of a frontal headache, photophobia, aching in the neck, sore throat, tender glands, profuse nasal discharge and nasal obstruction. Temperature 100.8°F. This individual used 174 gauze handkerchiefs during the first 24 hours of her cold. Examination showed that the mucous membrane of the nasal passages was hyperemic and on the right side marked swelling was present. A large amount of clear watery nasal discharge was seen. The

pharynx hyperemic, the glands at the angles of the jaw enlarged and tender. 7/22/30—The lacrimation, headache and sore throat had disappeared. The acuity of hearing was diminished. The nasal discharge less abundant and more mucoid. Examination: There was less swelling and hyperemia of the mucous membranes in the nose. A small amount of thick, sticky nasal discharge present on both sides. The throat only mildly injected. The glands at the angles of the jaw just barely palpable. 7/23/30—The nasal secretion was decreased in amount and was mucopurulent in type. Nasal obstruction intermittent. Examination showed better breathing space on both sides. Less congestion of the nasal mucous membranes. A small amount of sticky discharge. The throat was slightly injected. 7/24/30—Very slight nasal discharge; intermittent, with crusting. Examination normal except for a slight swelling of the inferior turbinates. Discharged. Atypical *D. pneumoniae*, Type II, present in cultures from the nasopharynx on the 5th day of the control period and on the 1st, 2nd and 3rd days after inoculation. Non-hemolytic *H. influenzae* present in two cultures during the control period. The aerobic cultures from the nasopharynx negative for hemolytic streptococci, type Beta. Anaerobic cultures showed Groups VII and IV organisms both before and after the inoculation. Cultures of the Berkefeld W filtrates were sterile. The white blood cell counts and urine normal throughout.

Volunteer II (Reentry).—Age 22 years. This subject again selected as a volunteer after an attempted inoculation 5 weeks previous to this entry. During the interim she had been free from upper respiratory disease. Five-day control period. Inoculated twice on 7/19/30 and once on 7/20/30 with Seitz filtrates of the nasopharyngeal washings from Volunteer XV. No symptoms or signs of a respiratory infection developed although in addition to the inoculation she was exposed to Volunteer XVII during the entire period. Non-hemolytic *H. influenzae* isolated in cultures from nasopharynx on 2nd, 3rd, 4th and 5th days of the control period and on the 3rd, 4th, and 5th days after inoculation. The aerobic cultures were negative for *Streptococcus haemolyticus*, type Beta, and *D. pneumoniae*. Anaerobic cultures showed Group IV organisms both before and after inoculation. The urine was normal throughout.

Volunteer XVIII.—Age 22 years. Tonsillectomy and adenoidectomy in 1913. Adenoidectomy in 1917. Influenza in 1918. Two colds during the past 2 years. Physical and nose and throat examinations normal. Two-day incubation period. Inoculated twice on 7/21/30 with Berkefeld W filtrates of the nasal washings from Volunteer XVII. 27 hours after the first inoculation, a sensation of dryness, fullness and of irritation in throat, a slight watery nasal discharge, partial nasal obstruction, sneezing, hoarseness and a frontal headache. 7/23/30—The nasal discharge more profuse and nasal obstruction more marked, with sore throat, frontal headache, aching in the neck and drowsiness. Examination showed that the nasal mucous membranes were moderately congested. A small amount of mucoid nasal discharge on both sides. The glands at the angles of the jaw enlarged, and slightly tender on the right. 7/24/30—Nasal discharge thick,

nasal obstruction intermittent. Sore throat persisted. The appearance of the nose and throat had not changed. 7/25/30—The nasal discharge was lessened in amount. Crusting was present. Nasal obstruction was intermittent. Examination showed less congestion and discharge. The posterior pharyngeal wall was hyperemic. 7/26/30—Very little nasal discharge. Nasal obstruction intermittent. 7/27/30—Very slight amount of nasal discharge, and practically no obstruction. 7/28/30—Discharged. Nose and throat examination was essentially normal. *D. pneumoniae*, Type IV, were isolated in cultures from the nasopharynx throughout the experiment. No *Streptococcus haemolyticus*, type Beta, or *H. influenzae* isolated in the cultures from the nasopharynx. Anaerobic cultures during the control period negative, but after inoculation Groups IV and VII organisms were isolated. White blood cell counts and urine normal throughout.

Volunteer XIX.—Age 23 years. Tonsillectomy and adenoidectomy in 1914. Influenza in 1924. Two colds during the past 2 years. The nose and throat examination essentially normal except for remnants of tonsil tissue on the posterior pharyngeal wall. Two-day control period. Inoculated twice 7/21/30 with Berkefeld W filtrates of nasal washings from Volunteer XVII. 24 hours later, sore throat and headache; very listless. Temperature 100°F. (rectal). 7/23/30—Had not slept well. A thin watery nasal discharge present, with intermittent nasal obstruction. Sore throat persisted. Temperature 100°F. (rectal). Examination showed no changes. 7/24/30—Sore throat remained unchanged. Backache was present. The nasal discharge more profuse and nasal obstruction more prominent. The subject was hoarse and complained of substernal pain; remained in bed the entire day. Temperature 100.4°F. (rectal). Examination showed hyperemia and swelling of the nasal mucous membrane. A small amount of mucoid nasal discharge. Ten hemorrhagic spots scattered over the uvula. The glands at the angles of the jaw enlarged and tender. 7/25/30—Felt much better. The nasal discharge had decreased and the nasal obstruction was intermittent. Temperature 100.1°F. (rectal). The nose and throat examination was unchanged. 7/28/30—Discharged. The nose and throat examination was normal. Nonhemolytic *H. influenzae* isolated in cultures from the nasopharynx on the 1st, 2nd, 3rd, 4th and 6th days after inoculation. Aerobic cultures of the nasopharynx negative for hemolytic streptococci, type Beta, and for *D. pneumoniae*. Anaerobic cultures were negative both before and after inoculation. White blood cell counts and urine normal during the entire period.

DISCUSSION

This study has to do with the transmission of upper respiratory infections (common colds) to human volunteers. Judging from their past histories, the individuals selected as subjects for the test varied moderately in respect to the number of natural "colds" which they had within the past 2 years, but no attempt was made to select them

on this basis. In evaluating the results, the strictness of quarantine and the selection of a season in the year when "colds" are not prevalent, are two considerations of great importance. We believe that by selecting intelligent volunteers who cooperated in observing strict isolation we have been able to fulfill the first of these requirements. The tests were made in Baltimore, Maryland, during a period (June and July, 1930) in which the attack rate of all upper respiratory infections was between one and two per hundred persons per week, a particularly low incidence.*

The nine subjects ill with natural colds, from whom the inoculum was obtained, were selected with care. Yeast infusion broth was used for washing the nasopharyngeal passages of the experimental subjects because Grinnell (18) has demonstrated that it facilitates the passage of certain viruses through filters. The results of our experiments agree with his findings. In the earlier experiments we were able to confirm the findings of previous investigators with regard to the transmission of upper respiratory infection with Berkefeld V filtrates. In each instance these filtrates contained Gram-negative filter-passing anaerobic organisms.

Seitz filters of the Uhlenhuth model and Berkefeld W filters were used in obtaining bacteria-free filtrates. Mudd (17) has demonstrated that the Uhlenhuth model of the Seitz filter does not permit the passage of test bacteria or certain dyes. In our experience neither filter has been permeable to the minute Gram-negative filter-passing organisms present in nasopharyngeal washings, and in all instances the filtrates from the Seitz and Berkefeld W filters remained sterile when cultivated in the test media. This is not due to an inhibitory or bactericidal effect of the filter materials for it is possible to cultivate Gram-negative anaerobes from a Berkefeld V filtrate in a medium to which large pieces of the Seitz filter discs have been added. Filtration was accomplished under negative pressure by use of the laboratory vacuum and not more than 30 minutes elapsed between obtaining the washings and completing the inoculations. The time element is of importance as the incitant may perish rapidly when removed from its natural environment.

* We were able to utilize data from a study of upper respiratory infection in a group of one hundred families through the kindness of Dr. V. A. Van Volkenburgh.

The studies of the aerobic bacterial flora in the nasopharynx showed no striking differences between the control period and the period of the upper respiratory infection. Certain of the volunteers did not harbor any of the organisms under observation; others were consistent carriers throughout the period of investigation. It is concluded that the particular aerobic bacteria studied played no part in the production of the upper respiratory infection.

Anaerobic cultivation of the Berkefeld V filtrates of the nasopharyngeal washings from the subjects ill with natural colds yielded positive results in all instances. No constant predominating organism was found.

Nineteen individuals acted as subjects and twenty inoculation tests were performed. In the first ten the volunteers were divided into pairs, one of each pair receiving Berkefeld V filtrates and the other Seitz filtrates of the nasopharyngeal washings from individuals ill with natural "colds." Two of the subjects receiving Seitz filtrates and two receiving Berkefeld V filtrates developed "colds." These tests confirmed the observation of earlier observers in regard to the transmission of "colds" by means of Berkefeld V filtrates and also demonstrated that the infection can be transmitted by bacteria-free filtrates.

In the second group of ten tests serial transmission of the infection through two and four passages was accomplished with bacteria-free filtrates. In the first instance the source of infectious material was Volunteer X whose infection developed subsequent to inoculations with Berkefeld V filtrates from the nasopharyngeal washings of an individual ill with a natural "cold." The affection was transmitted in serial passage from Volunteer X through Volunteers XI and XII by means of Seitz filtrates. The source of material for the second serial transmission test was Patient I and the transfer of her "cold" to Volunteer XIV and the subsequent serial passage of this infection through Volunteers XV and XVII was accomplished with Seitz filtrates. A fourth generation passage of the affection from Volunteer XVII to Volunteers XVIII and XIX was made with Berkefeld W filtrates. Thus two single transfers and serial transmission through two and four generations have been made with bacteria-free filtrates and the earlier observation in regard to transmission of "colds" with Berkefeld V filtrates confirmed.

In all, eleven out of twenty inoculations resulted in upper respiratory infection. During the 7 week period in which the inoculations were done the prevalence of upper respiratory infection in a group of one hundred families under constant and close observation was at the rate of 2.13 per cent per week. With this rate of prevalence we might expect to have had about 5 per cent of our subjects develop natural "colds" within the period of observation which in each individual was about 10 days, if they had been at large in their normal surroundings. However, this rate of prevalence would naturally be greatly decreased by the strict isolation of the volunteers so that it would be difficult to determine the expected rate of prevalence. This is to be compared with the actual occurrence of eleven colds in nineteen people, and moreover, all colds occurred within 3 days of inoculation with no instance of a cold preceding inoculation or following it after an interval of 3 days.

In general the upper respiratory affections developed by the volunteers subsequent to inoculation were attended at their inception by mild constitutional disturbances. In six of the successful transmissions there was a slight fever during the initial stages of the infection. In three no febrile reaction was observed. The remaining two developed slight fever late in the course of the disturbance. The incubation period (as measured from the time of the first inoculation to the onset of symptoms) varied from 20 to 70 hours.

The earliest symptoms consisted of sensations of fullness, dryness and irritation in the nasopharynx. Some subjects complained of an actual sore throat. Pharyngitis was generally present and appeared to be associated with the spread of the virus as no changes in the aerobic flora could be demonstrated in the nasopharynx. In the early stages of the infection, the nasal discharge was thin and watery but it varied greatly in amount. The objective changes in the nose consisted of hyperemia, swelling of the mucous membrane and nasal discharge. These signs were found to change rapidly within short periods of time. The frequent nose and throat examination aided us in diagnosing the affection, since definite evidence of nasopharyngeal abnormality was required before an experiment could be considered positive. None of the volunteers developed complications secondary to their upper respiratory infections. This, we believe, can be attrib-

uted to the time of the year in which the experiments were conducted, and to the fact that the subjects were protected by quarantine against potentially pathogenic microorganisms. A close check was kept upon each volunteer after leaving the hospital and in no instance did an acute exacerbation of the infection occur. There were no abnormal findings in the urines of the subjects during the experimental period and the records of the white blood cell determinations do not show any significant changes attributable to the presence of infection.

Following the inoculations two of the volunteers developed short, but nevertheless distinct attacks of diarrhoea accompanied by malaise and prostration. One was suffering from an experimental upper respiratory infection and the other did not show any signs of nasopharyngeal involvement. In one case a definite leukopenia developed and in the other the white blood cell count was decreased but did not reach a leukopenic stage. Stool cultures from both individuals showed a normal flora. At the time there were no other individuals ill with a similar disease in the hospital. The clinical picture presented by these two volunteers closely resembles the influenzal syndromes which are frequently observed during waves of upper respiratory infection. We present these two interesting cases but we do not feel that any definite conclusions can be drawn from them at this time.

CONCLUSION

Experimental upper respiratory infections similar to "common colds" were transmitted singly and in series through two and four passages in nine out of fifteen persons, by intransal inoculations with bacteria-free filtrates of nasopharyngeal washings obtained from individuals ill with natural "colds." These observations conform with those reported by previous workers and lend further support to the view that the incitant of the "common cold" is a filtrable virus.

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BIBLIOGRAPHY

1. Kruse, W., *Münch. med. Woch.*, 1914, **61**, 1547.
2. Foster, J. B., Jr., *J. Am. Med. Assn.*, 1916, **66**, 1180; *J. Infect. Dis.*, 1917, **21**, 451.

3. Dold, H., *Münch. med. Woch.*, 1917, **64,** 143.
4. Schmidt, P., *Deutsch. med. Woch.*, 1920, **46,** 1181.
5. Williams, A. W., Nevens, M., and Gurley, C. R., *J. Immunol.*, 1921, **6,** 5.
6. Olitsky, P. K., and McCartney, J. E., *J. Exp. Med.*, 1923, **33,** 427.
7. Robertson, R. C., and Groves, R. L., *J. Infect. Dis.*, 1924, **34,** 400.
8. Shibley, G. S., Hanger, F. M., and Dochez, A. R., *J. Exp. Med.*, 1925, **43,** 415.
9. Mills, K. C., Shibley, G. S., and Dochez, A. R., *J. Exp. Med.*, 1928, **47,** 193.
10. Dochez, A. R., Shibley, G. S., and Mills, K. C., *Proc. Soc. Exp. Biol. and Med.*, 1929, **26,** 562.
11. Shibley, G. S., Mills, K. C., and Dochez, A. R., *Proc. Soc. Exp. Biol. and Med.*, 1929, **27,** 59.
12. Dochez, A. R., Shibley, G. S., and Mills, K. C., *J. Exp. Med.*, 1930, **52,** 701.
13. Shibley, G. S., Mills, K. C., and Dochez, A. R., *J. Am. Med. Assn.*, 1930, **95,** 1553.
14. Long, P. H., and Doull, J. A., *Proc. Soc. Exp. Biol. and Med.*, 1930, **28,** 53.
15. Avery, O. T., *J. Am. Med. Assn.*, 1918, **71,** 2050.
16. Bourn, J. M., *J. Prev. Med.*, 1928, **2,** 441.
17. Mudd, S., in Rivers, T. M., Filterable viruses, The Williams and Wilkins Company, Baltimore, 1928, 59.
18. Grinnell, F. B., *J. Bact.*, 1929, **18,** 175.