

EXPERIMENTAL HUMAN INOCULATIONS WITH
FILTERED NASAL SECRETIONS
FROM ACUTE CORYZA

ROBERT C. ROBERTSON
AND
ROBERT L. GROVES

*From the Department of Pathology and Bacteriology, University of Illinois,
College of Medicine, Chicago*

A review of the literature on this subject reveals the great divergence of results obtained by various investigators.

Kruse¹ succeeded in producing colds experimentally with a filtrate obtained from the nasal secretions of persons suffering with acute coryza.

Foster² succeeded in confirming the experiments of Kruse. Cultivating the filtrate by the method of Noguchi, he obtained a minute active organism visible with the dark field, which he states he successfully transplanted to the 4th generation, and which when then inoculated into the nostrils of 11 volunteers produced acute coryza in all.

Schmidt³ from a series of 196 filtrate inoculations from 16 different subjects suffering with coryza concludes that his investigations do not support the filtrable virus theory of "colds."

Branham and Hall⁴ state that in attempting to cultivate filtrable viruses from nasopharyngeal secretions in colds and influenza on the various mediums employed by them, no bodies were found in the cultures which could not be found also in those from normal persons, in controls, in all simple mediums examined, and on blank slides. This work does not support the work of Foster,² who contends that definite bodies were found when cultivated according to Noguchi's method.

Bloomfield⁵ states that in a review of the literature he has found no convincing evidence that any known organism is the primary cause of colds, and cultural studies in his report fail to show in uncomplicated

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¹ Münch. med. Wchnschr., 1914, 61, p. 1547.

² Jour. Infect. Dis., 1917, 21, p. 451; Jour. Am. Med. Assn., 1916, 66, p. 1180.

³ Deutsch. med. Wchnschr., 1920, 46, p. 1181.

⁴ Jour. Infect. Dis., 1921, 28, p. 143.

⁵ Bull. Johns Hopkins Hosp., 1921, 32, p. 121.

cases any variation in the flora which would enable one to select any organism or group of organisms as the cause of common colds.

Olitsky and Gates⁶ have isolated an organism, *Bacterium pneumosintes*, from the nasopharynx of patients suffering from uncomplicated influenza. This when cultivated and injected into the trachea and nasopharynx of rabbits and guinea-pigs causes the clinical symptoms of influenza and seems to confer a certain degree of immunity to the rabbits, in some cases as long as 14 months. The organism is filtrable in the sense that it will pass through an ordinary porcelain filter, but it can be seen microscopically.

Lister⁷ cultivated on Smith-Noguchi medium an organism corresponding to *Bacterium pneumosintes* and recovered it in nasopharyngeal washings from a volunteer who was taken sick with influenza following inoculation.

Olitsky and McCarty⁸ conclude that the nasal secretions of a person suffering with acute coryza when taken from 3-18 hours after the onset contain a filter passing incitant and that common colds and epidemic influenza are separate and distinct diseases. They failed to find a specific exciting cause of coryza.

Of the factors believed to be the exciting cause of acute coryza by the various investigators, a filtrable virus appeared to be the most promising of positive results. It was our endeavor therefore to repeat as well as to extend experimental work in this field, with a view to acquiring more definite data, especially in connection with the nature of any virus which might be present.

In selecting cases for these experiments we limited ourselves to uncomplicated coryza which appeared during the fall, winter, and spring; and of these we endeavored to select the more virulent type, therefore apparently the most infectious.

The nasal secretions of persons suffering with such coryza were collected, from 6 to 144 hours after the onset, in a container which had been thoroughly sterilized and kept from contamination so far as possible; they were mixed with sterile salt solution in varying dilutions, from 1:10 to 1:50, and the mixture was shaken with glass beads in a small jar until homogeneous and free from lumps of mucus.

A record was made of clinical data with special reference to (a) history of recent exposure to acute coryza; (b) severe chilling as the

⁶ Jour. Exper. Med., 1922, 35, p. 1.

⁷ South African Medical Record, 1922, 22, p. 434.

⁸ Jour. Exper. Med., 1923, 38, p. 427.

result of temperature changes or insufficient clothing; (c) evidence of contagion among associates. Smears of the secretions were then made and stained by Gram method and methylene blue.

Aerobic blood-agar plates were inoculated with a loop of this material, examined at the end of 24 and 48 hours, and the organisms identified.

The solution was then passed through a Berkefeld filter which was impermeable to ordinary cocci using tap water pressure—a procedure never taking more than one hour—and at the completion of which, smears and aerobic and anaerobic blood-agar cultures were made of the filtrate. In every case these cultures from the filtrate—both aerobic and anaerobic—remained sterile for 96 hours. On all of the smears made from the filtrate were found coccoid bodies, apparently those which were believed by Foster² to be the specific exciting cause of acute coryza.

These coccoid bodies were quite small, though visible under oil immersion, round or slightly flattened, quite distinct in outline, and possessed a marked tendency to occur in pairs, although they also appeared singly or in clusters. They did not appear to possess true motility and retained the Gram stain. They could be demonstrated in filtrates from the nasal washings of normal persons and on stained clean glass slides as readily as in the filtrates from the nostrils of persons suffering with acute coryza. Beyond the mediums mentioned no attempt was made by us to cultivate these bodies, for we found no reason to believe that they played a part in the etiology of acute coryzas, or indeed were organisms at all.

Inoculations with the filtrate were made into the nostrils of volunteers free from coryza or catarrhal affections as soon after filtration as possible. In no case did this time exceed 24 hours after the collection of the secretions, which in the meantime were kept in the icebox. For inoculation purposes, we found it convenient to use sterile, drawn glass tubes plugged at one end with cotton, in which the filtrate was aspirated and the drawn end sealed in the flame. At the time of inoculation this sealed end was broken off, sterilized by the flame, and the liquid sprayed over the nasal mucosa of the volunteer. About 1 c. c. of the filtrate was placed in each nostril by this method.

All infections of the respiratory tract following within 12 days of the inoculation were regarded as positive even though they did not later develop into a coryza. The inoculated person reported the infection as

soon as possible, and the clinical signs, as well as the history of exposure and contagion among associates, were recorded. When no symptoms had appeared by the end of the 12th day, the inoculation was recorded as negative.

The volunteers inoculated during these experiments were faculty members, students, or employees of the University of Illinois, College of Medicine or College of Dentistry, whose daily work brought them in contact with many persons from whom they, unknown to themselves, might have contracted coryza, or transmitted it to them.

In summarizing the observations, it is found that the onset dated back to exposure due to chilling of the body in 2 cases (18.18%), and to other coryzas in 1 case (9.09%). There was a negative history of exposure in 8 cases (72.72%). There was evidence of contagion among associates in 3 cases (27.27%).

Bacteriologically, the incidence of the various organisms which occurred in the unfiltered secretions is:

Staphylococcus albus in 9 cases (81.81%)
 Micrococcus catarrhalis in 4 cases (36.36%)
 Streptococcus nonhemolyticus in 4 cases (36.36%)
 Streptococcus viridans in 3 cases (27.27%)
 Bacillus influenzae in 1 case (9.09%)
 Diphtheroids in 1 case (9.09%)
 Pneumococcus (IV) in 1 case (9.09%)
 Streptococcus hemolyticus in 1 case (9.09%)

The incidence of 2 organisms in the secretions is:

Staphylococcus albus and micrococcus catarrhalis in 2 cases (18.18%)
 Staphylococcus albus and Streptococcus viridans in 2 cases (18.18%)
 Staphylococcus albus and Streptococcus hemolyticus in 1 case (9.09%)
 Staphylococcus albus and Streptococcus nonhemolyticus in 1 case (9.09%)
 Streptococcus viridans and Streptococcus nonhemolyticus in 1 case (9.09%)

The incidence of three organisms in the secretions is:

Staphylococcus albus, Micrococcus catarrhalis, and diphtheroids, in 1 case (9.09%)
 Staphylococcus albus, Streptococcus nonhemolyticus and B. influenzae in 1 case (9.09%)
 Staphylococcus albus, Micrococcus catarrhalis, and pneumococcus (IV) in 1 case (9.09%)

The results of the 100 human inoculations are positive for bronchitis, 1 case (1%); coryza, 1 case (1%); influenza, 1 case (1%); and laryngitis, 2 cases (2%). There were 95 negative cases, or 95%, free from any respiratory infection following the inoculation.

TABLE 1
RESULTS OF EXPERIMENTAL INOCULATIONS

Subject			Secretions						Inoculations of Volunteers				Bacteriology		
No.	Onset	Predisposing Factors	Course	Evidence of Contagion	Collected	Bacteriology	Dilution	No. of Volunteers	Date	No. of Negatives	Number	Onset	Predisposing Causes	Course	Evidence of Contagion
I	Nov. 2, 1921	None	Mod-erate	None	Nov. 3, 1921 (30 hours from onset)	Staphylococcus albus, nonhemolytic streptococcus	25x	6	Nov. 4, 1921	6	0				
II	Dec. 12, 1921	Chilled while traveling on railroad train, night of Dec. 11	Mod-erate	All of room-mates (3 in number) developed coryza within 7 days	Dec. 14, 1921 (36 hours from onset)	Staphylococcus albus, Micrococcus catarrhalis, Pneumococcus (IV)	25x	6	Dec. 14, 1921	5	1	Dec. 17, 1921	None	Mild laryngitis	None
III	Dec. 13, 1921	Evening of Dec. 12 studied with friend suffering with acute coryza	Severe	None	Dec. 14, 1921 (12 hours from onset)	Staphylococcus albus, Streptococcus viridans	10x	6	Dec. 15, 1921	5	1	Dec. 15, 1921 1½ hours after inoculation	None	Mild influenza	None
IV	Jan. 3, 1922	None	Mod-erate	None	Jan. 3, 1922 (6 hours from onset)	Streptococcus viridans, Streptococcus nonhemolyticus	25x	7	Jan. 4, 1922	6	1	Jan. 7, 1922	Fatigue from loss of sleep	Mild laryngitis	None
V	Feb. 4, 1922	None	Severe	Wife and 2 children developed similar coryza within 7 days	Feb. 10, 1922 (144 hours from onset)	Staphylococcus albus, Streptococcus nonhemolyticus, B. influenzae	25x	7	Feb. 11, 1922	7	0				
VI	Feb. 12, 1922	Chilling because of insufficient bed clothing night of Feb. 11	Severe	None	Feb. 13, 1922 (24 hours from onset)	Streptococcus nonhemolyticus	30x	4	Feb. 14, 1922	4	0				
VII	Mar. 21, 1922	None	Severe	None	Mar. 22, 1922 (30 hours after onset)	Staphylococcus albus, Streptococcus hemolyticus	50x	10	Mar. 23, 1922	8	2	No. 5 on March 24 No. 8 on March 23	None	Mild coryza Mild bronchitis	None
VIII	Apr. 3, 1922	None	Severe	None	April 4, 1922 (12 hours from onset)	Staphylococcus albus, Micrococcus catarrhalis	25x	20	April 5, 1922	20	0				
IX	Apr. 17, 1922	None	Severe	None	April 18, 1922 (18 hours from onset)	Staphylococcus albus, Micrococcus catarrhalis	40x	14	April 18, 1922	14	0				
X	Oct. 17, 1922	None	Severe	Two sons developed coryza of similar type with in 5 days from onset	Oct. 20, 1922 (60 hours from onset)	Staphylococcus albus, Micrococcus catarrhalis, Diptheroids	25x	10	Oct. 20, 1922	10	0				
XI	Dec. 14, 1922	None	Severe	None	Dec. 15, 1922 (12 hours from onset)	Staphylococcus albus, Streptococcus viridans	30x	10	Dec. 15, 1922	10	0				

DISCUSSION

Because of the small number of positive results following the inoculations, we found it unnecessary to employ controls, and the variation in the 5% of cases recorded as positive convinces us that these were the result of causes entirely independent of the inoculations. In any group of persons selected at random during a time when an endemic of colds is present, a certain small percentage will develop some upper respiratory infection within a period of 12 days. This we believe fully explains the cases which we recorded as positive.

Though extensive experiments have been made on the bacteriology of acute coryza, the results obtained have been far from satisfactory. As our experiments were planned primarily to establish the status of a filtrable virus in their etiology, no human inoculations were attempted with any of the cultivated organisms.

In all cases examined a marked reduction of the bacterial flora occurred in the secretions with the predominance of one organism during the onset and early stages of the attack. When the secretion became purulent, usually from 36 to 48 hours from the onset, bacterial examination revealed a marked increase of the flora over the normal flora of health still with the predominance of one organism, usually *Staphylococcus albus*. When the secretions began to diminish, the bacterial flora diminished and gradually returned to that of health.

We believe this to be a point of some significance when considered with the negative results obtained on filtrate inoculation. Presumably it is an indication that the exciting factor is a specific, nonfiltrable organism which in some manner decreases the nasal flora during the onset and early stages of the attack, while its elimination during the course of the attack, together with its depressant action on the mucosa, permits a marked increase over the normal flora (still, however, with one predominating organism) and during convalescence a gradual return to the flora of health.

Although from bacteriologic examinations no satisfactory proof has as yet been produced which shows that coryzas are infectious, all clinical and epidemiologic manifestations point in this direction. One has but to note the occurrence of endemics and epidemics of acute coryza in civilized communities—the absence of which has often been noted in the arctic regions by explorers—to be convinced that the specificity of these infections is as clearly apparent as in many of the acute infectious diseases of established etiology.

Predisposing causes, such as seasonal changes, lowered resistance, exposure to cold and moisture, and individual anatomic variations have been pointed out by many investigators and must be kept in mind at all times, as should also the clinical variations observed from the type with but slight serious discharge and no systemic effects to the virulent type closely resembling influenza.

That coryzas frequently play a large part as predisposing causes of other acute or chronic infectious respiratory diseases cannot be questioned. This inter-relationship is frequently seen in influenza, pneumonia, and tuberculosis, in which the onset is said to date back to "a cold," and for this reason their prevention and treatment should play an increasingly large part in modern preventive medicine.

SUMMARY

In this series of experiments, nasal secretions were secured from 11 persons suffering with acute uncomplicated coryza. After being diluted and passed through a Berkefeld filter, these secretions were sprayed onto the nasal mucosa of 100 volunteers.

The experiments presented no convincing evidence indicative of a filter passing organism as the exciting factor in acute coryza. We believe the cases recorded as positive to be the result of factors independent of the inoculations.

During an attack of coryza definite variations were noted in the bacterial flora of the secretions. During the onset and early stages of the attack, there was a marked diminution of the total bacterial flora, with an equally marked predominance of one of the normal habitants—usually *Staphylococcus albus*.

During the purulent stage of the attack, a marked increase of all organisms over the normal flora of health was observed, although the predominance of one organism still remained. The later stages of the attack were marked by a gradual return to the normal flora of health.