THE EFFECTS OF EXPOSURE TO COLD UPON EXPERIMENTAL INFECTION OF THE RESPIRATORY TRACT.*

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Exposure to cold has long been considered an important factor in the incidence of many respiratory diseases. Even with our increasing knowledge of the predominating part of bacterial infection, the belief is still general among clinicians that chilling of the body exerts a predisposing influence of considerable importance.

In reviewing the general subject of the effect of atmospheric conditions upon bodily health and efficiency, the New York State Ventilation Commission was impressed with the comparative lack of accurate data concerning the physiological effect of low temperatures, the majority of recent observers having interested themselves mainly with the study of various degrees of heat.

Experimental studies of the influence of cold as well as of heat have therefore formed a part of the work of the Commission for the past 2 years. The experiments here reported constitute a portion of these studies.

EXPERIMENTAL.

The organism chosen for inoculation was *Bacillus bovisepticus*, or snuffles bacillus, which belongs to the hemorrhagic septicemia group and is pathogenic for the rabbit. It is a small, round bacillus with a marked tendency to bipolar staining, particularly in blood films made from animals dying from infection caused by it. The disease produced under natural conditions is generally a true septicemia, but in many cases is localized in the upper respiratory passages,

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when it is commonly known as snuffles. This condition is characterized by a typical discharge of yellowish mucus from the nostrils and may terminate in recovery or in a fatal pneumonia with or without general septicemia.

It will thus be seen that the conditions produced in the rabbit by this bacillus are similar to some of the respiratory infections in man, such as pneumonia and influenza. The organism was selected for use in our work because of this fact and because of the relative difficulties which have attended previous experiments with acute respiratory infections, particularly those caused by the pneumococcus.

Experiment 1.—This experiment was conducted during the late winter months of 1915 and 1916. Thirty-seven experimental rabbits were used in thirteen separate series of from two to four animals, each with an equal number of controls.

The experimental rabbits were kept in an incubator at warm temperatures for periods varying from 1 day to 1 week. In eleven series this temperature was between 85–90° F., and in two it varied from 70–76° F. and 63–78° F., respectively, being, however, at the higher level most of the time. The animals were then inoculated by spraying the nasal mucous membrane or the throat with a small amount of the live culture of *Bacillus bovisepticus*, emulsified in salt solution. After inoculation they were immediately chilled by exposing them to the outside weather for periods varying from ½ to 3 hours and at a temperature of 20–56° F. In the majority of the experiments the fur of the animals was wet with water at about body temperature, to facilitate chilling upon exposure. The details of temperature, time of exposure, method of inoculation, etc., are shown in the table under each experiment.

The control animals, also thirty-seven in number, were kept constantly at a temperature of 65–70° F. and were inoculated at the same time and in the same manner as the experimental animals of the corresponding series. None of these were wet.

Results.—Of the experimental rabbits five died of pneumonia, and one of general septicemia, while nine were made ill, but recovered. Of the controls three died and six others were infected, but recovered. In every case when death resulted, the animal was autopsied, the character of the lesions noted, and the specific organism isolated from the heart's blood.

It will thus be seen that of the experimental animals fifteen, or 40 per cent, reacted, and only nine, or 24 per cent, of the controls. It is also worthy of note that this difference in reaction between the experimental and the control groups was harmoniously distributed

throughout the entire thirteen series of experiments. In only two series (Nos. 11 and 12), did the number of reactions in the control group exceed that of the experimental animals. The details of this experiment are shown in Table I.

It seemed desirable to note the effect of reversing the conditions; that is, to determine the result of a change from a cold to a hot temperature. It was possible to conduct only two series in this experiment.

Experiment 2.—Four animals were kept at out of door temperature for 2 days, and after inoculation as in the previous experiment were placed in an incubator at 78–80°F. for from 6 to 12 days. A similar number of controls, inoculated in the same way, were kept constantly at room temperature (65–70° F.).

Results.—Of the experimental animals all reacted, of which one died. Of the controls only two reacted, one of which also died. A detailed description is given in Table II.

From the results obtained in the two foregoing experiments it seemed desirable to attempt one further experiment in which three modifications of the experimental conditions were considered together. (1) A change from outside temperature (59° F.) to incubator temperature (80° F.), (Series 16). (2) A change from room temperature (65–70° F.) to incubator temperature (80° F.), then down to a low temperature (50–60° F.), and from that back to room temperature (65–70° F.), (Series 17). (3) A modification of the conditions of Experiment 1; namely, chilling from a high temperature (80° F.) to a low one (57° F.), and then kept at 65-70° F. (Series 18).

Experiment 3.—All three series of this experiment were carried on simultaneously with two animals in each series, and with one set of two control animals which were kept continuously at room temperature (65–70° F.).

Results.—In Series 16, subjected to a change from low to high temperature, both animals died of pneumonia. In Series 17, subjected to several changes from room temperature, to high, then to low, and back to room temperature, both animals reacted, of which one died of pneumonia. In Series 18, subjected to a change from high to low temperature, only one animal reacted slightly with snuffles. Neither of the two controls reacted. Table III shows these results in detail.

TABLE I.

Series.	Heated and exposed.			Controls, kept at room temperature (65–70°F.).		
	No. of rabbits.	Reactions.		No. of rabbits.	Reactions.	
		Died.	Sick.		Died.	Sick.
1	4 24 hrs. at 87°F.; chilled to 45°F. for 1 hr. 2, fur wet; 2, fur dry. 2, nose; 2, throat.	2 Pneumonia. 1, fur wet; 1, fur dry. Death in 48 hrs.	1 General reaction; could hardly move; no appetite. Recovered.	4	0	1 General reaction. Recovered.
2	2 1 wk. at 85–89°F.; chilled to 40°F. for 1½ hrs. Fur dry. 1, nose; 1, throat.	0	1 Snuffles. Recovered.	2	0	0
3	2 1 wk. at 85–89°F.; chilled to 38°F. for 3 hrs. Fur dry. 1, nose; 1, throat.	0	1 Snuffles. Recovered.	2	1 Pneumonia. Death in 1 wk.	0
4	4 1 wk. at 85–90°F.; chilled to 38°F. for 1½ hrs. Fur dry. 2, nose; 2, throat.	0	0	4	0	0
5	4 1 wk. at 85–90°F.; chilled to 56°F. for 1 hr. Fur dry. 2, nose; 2, throat.	0	0	4	0	0
6	4 1 wk. at 85–90°F.; chilled to 20°F. for ½ hr. Fur dry. 2, nose; 2, throat.	1 Snuffles; septicemia. Death in 10 days.	0	4	0	0

7	4 1 wk. at 85–89°F.; chilled to 45°F. for 2 hrs. Fur dry. 2, nose; 2, throat.	0	1 Snuffles. Recovered.	4	0	1 Snuffles. Recovered.
8	3 1 wk. at 85–90°F.; chilled to 40°F. Fur wet.	0	2 Snuffles. Recovered.	3	0	0
9	2 3 days at 70–76°F.; chilled to 25°F. for ½ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 2 days. Inoculated in throat.	1 Snuffles. Inoculated in nose. Recovered.	2	2	0
10	2 3 days at 85–93°F.; chilled to 52°F. for ½ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 11 days. Inoculated in nose.	0	2	0	0
11	2 24 hrs. at 82–87°F.; chilled to 27°F. for ½ hr. Fur wet. 1, nose; 1, throat.	0	0	2	0	1 Snuffles. Recovered. Inoculated in nose.
12	2 24 hrs. at 85–90°F.; chilled to 31°F. for ½ hr. Fur wet. 1, nose; 1, throat.	0	1 Snuffles. Severe reaction. Recovered. Inoculated in nose.	2	0	2 Snuffles. Mild reaction. Recovered.
13	2 4 days at 63–78°F.; chilled to 32°F. for ⅓ hr. Fur wet. 1, nose; 1, throat.	1 Pneumonia. Death in 3 days. Inoculated in throat.	1 Snuffles. Recovered. Inoculated in nose.	2	0	1 Snuffles. Recovered. Inoculated in nose.
Total	37	6	9	37	3	6

TABLE II.

Series.	Kept cold in the open, then heated in incubator.			Controls, kept at room temperature (65–70°F.).		
	No. of rabbits.	Reactions.		No. of rabbits.	Reactions.	
		Died.	Sick.		Died.	Sick.
14	2 2 days at 41°F.; 12 days at 80°F. 1, nose; 1, throat.	0	2 Snuffles. 1, inoculated in nose, reacted in 4 days; 1, inoculated in throat, reacted in 6 days.	2	0	1 Reacted in 12 days; Inoculated in nose.
15	2 2 days at 35–40°F.; 6 days at 78°F.	1 Pneumonia. Inoculated in throat.	1 Snuffles. Inoculated in nose.	2	1 Pneumonia. Inoculated in throat.	0
Total	4	1	3	4	1	1

TABLE III.

Experimental.									Controls, kept at room temperature (65–70° F.).		
Series 16.			Series 17.			Series 18.					
48 hrs. at 59° F.; inoculated and kept at 80° F. 1, nose; 1, throat.			48 hrs. at 65–70° F.; inoculated; 24 hrs. at 80° F.; 4 days in open (50–60° F.); then at room temperature (65–70° F.).			2 days' incubation (80° F); inoculated; fur wet; 20 min. at 57° F.; then kept at 65–70° F.					
No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.	No. of rabbits.	Died.	Reacted.
2	2 Pneumonia.	0	2	1 Pneumonia. Death in 6 days. Inoculated in nose.	1 Mild attack of snuffles in 7 days. Inoculated in throat.	2	0	1 Snuffles in 3 days. Inoculated in throat.	2	0	0

DISCUSSION.

Other experimental studies of respiratory disease have been numerous, but few of these deal directly with the associated problem of the effect of chill with which we are at present concerned.

Lipari (1) in 1888 reports the results of intratracheal injections of pneumonic sputum in guinea pigs and rabbits which, after exercise by running, were chilled either by cold baths at 3°C. or by the application of ether to the skin after the animals had been shaved. Of eight chilled animals six died, while only two of twelve controls were affected.

Lode (2) in 1897 shaved guinea pigs over one-half to two-thirds of the body, exposed them to heat (37°C.) for half an hour, and then chilled them in draughts at open windows. Using Buchner's apparatus he exposed them to the spray of cultures of Friedländer's bacillus. Of eleven chilled animals seven died, while only three of eleven controls succumbed. In a similar inhalation experiment with tubercle bacilli, of five chilled animals all died, but only two of four controls. He also used Friedländer's bacillus, cholera bacillus, and *Staphylococcus aureus* subcutaneously in his series, as well as anthrax with a few chickens and rats. Altogether, including both inhalation and subcutaneous methods, he reports that forty-six of fifty-four chilled animals (85 per cent) died, and only nine of forty-five controls (20 per cent).

Pasteur's (3) experiments upon chickens made susceptible to anthrax by partial immersion in cold water are well known, but these as well as others not dealing with direct respiratory infection need not be considered here.

Chodounský (4), who has covered the whole field of the relation between exposure to cold and disease perhaps more extensively than any writer, comes to the conclusion that such exposure plays no part in determining the incidence of any of the various so called cold diseases. As a part of his contention he offers considerable experimental evidence concerning respiratory infections.

The experimental animals in Chodounský's studies were chilled by exposure to ice baths and cold draughts until their rectal temperature was lowered from 1–7°C. He employed several kinds of animals and several different pathogenic bacteria.

Virulent pneumococcus cultures by inhalation were used with one rabbit and one guinea pig with similar animals for controls. The rabbit was infected and died, but the guinea pig as well as the two controls survived.

Intravenous injections of pneumococci were also employed with practically identical results in the chilled and control animals.

Virulent cultures of Friedländer's bacillus were used by intratracheal injections in dogs and by inhalation with Buchner's apparatus in guinea pigs. Six dogs and two guinea pigs with a similar number of controls were employed. Of the dogs two experimental animals and three controls were infected; of the guinea pigs one each of the experimental animals and controls.

Chodounský also used cultures of Friedländer's bacillus attenuated by heat to determine whether these cultures might infect chilled animals. Intratracheal injections in four experimental dogs and four controls failed to infect any. Later, one of the controls was injected with a virulent culture and promptly succumbed. Chodounský also experimented with intravenous injections of pneumococcus in guinea pigs, with intraperitoneal injections of Friedländer's bacillus in rats, and with subcutaneous injections of diphtheria bacilli either alone or in combination with streptococcus, and in all these found no difference in reaction between the chilled and control animals.

More interesting and perhaps even more instructive are the results of Chodounský's self-experimentation. He describes himself as a man 57 years of age, subject to catarrh, frequent bronchitis, and lumbago. He reports in detail the results of twenty-seven separate experiments upon the effect upon himself of severe exposure to cold. After hot baths, after ice cold baths, and even when suffering from an acute coryza, he exposed himself naked to cold air and draughts at temperatures from 3–12°C. for periods of time varying from 20 minutes to 1 hour. Such exposure often caused severe shaking chills, but no permanent effect of any kind and no symptoms of any of the pathological conditions usually ascribed to exposure to cold.

Recently Kline and Winternitz (5), in the course of their studies of experimental pneumonia in rabbits, found that in the case of animals which were ex-

posed to cold (4°C.), after the ingestion of alcohol every day for periods varying from 1 to 9 days, four out of seven died, two of which showed intense laryngitis, tracheitis, and bronchitis, and one a bronchopneumonia. One other animal was killed at the end of 3 days and showed patchy congestion in the lungs. In the two remaining animals cultures of pneumococcus were injected intratracheally and death occurred in both, with lesions in the lungs and upper air passages. Pneumonia rarely occurred after such injections in animals not exposed to the effects of cold and of alcohol.

The weight of the experimental evidence thus reported appears to be against Chodounský's contention that exposure to cold has no influence upon respiratory disease, and with this evidence the results of our experiments are in accord.

The method of action of these secondary factors in infection is open to discussion. The lowering of general or specific resistance to infection may be considered. It has been shown by a number of observers including ourselves (6) that the production of such antibodies as hemolysins and agglutinins is affected by atmospheric temperature, especially, however, by heat. On the other hand, changes in the local resistance of the mucous membranes of the upper air passages may be more important. That definite circulatory changes do occur in the upper air passages as a result of varying atmospheric temperature has been observed in animals and also in clinical experiments upon human subjects by one of us (7) in collaboration with Cocks, which corroborated similar observations by Hill and Muecke (8). In these experiments the results appeared to be due to the direct action of air upon the mucous membranes and not to a reflex from the surface of the body. The possible concern of vagus control in this action, as suggested by Kline and Winternitz (5), is worthy of serious consideration. The fact that infection of the normal respiratory tract is experimentally difficult tends to emphasize the probable importance of the local changes in the mucous membranes favoring the entrance of bacteria as the main predisposing factor in the problem under consideration.

CONCLUSIONS.

1. Respiratory infection of rabbits with *Bacillus bovisepticus* (snuffles) is favored by chilling the animals after they have been accustomed to heat.

2. The character of this disease, which occurs frequently in rabbits under natural conditions, makes the application of the experimental results to similar respiratory conditions in man less open to objection than in similar experiments with other infections.

3. The weight of experimental evidence, including our own, does not justify the elimination of exposure to cold as a possible though secondary factor in the incidence of acute respiratory disease.

4. From the limited data of our last two experiments it is suggested that any marked change of temperature predisposes rabbits to this infection, the severity of which varies with the amount of change, and that a change from low to high temperature has an even more marked effect than that from high to low.

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