13262

Effect of Heat, Cold, Fatigue and Alcohol on Resistance of Mice to Human Influenza Virus.

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In preliminary work it was found that age was an important factor in the resistance of ordinary white mice to the influenza virus. Animals 4 to 6 weeks of age died regularly between 3 to 6 days after 0.05 cc of a 5% influenza filtrate was instilled into their nostrils. Older animals survived the same treatment for longer periods, in some cases as long as 17 days. Studies were outlined in an attempt to learn the effect of heat, cold, fatigue, and alcohol upon the resistance of mice to the virus. However, in early experiments it was found that old animals did not tolerate heat, cold and fatigue; therefore, 4- to 6-weeks-old mice were regularly employed.

The inoculum consisted of 0.05 cc of a 1:100,000 dilution of mouse lung tissue infected with the PR8 strain of influenza virus. It was prepared by grinding a 5% tissue suspension in broth of pH 7.2, centrifugating at 1600 rpm for 10 minutes and then passing the supernatant fluid through a Berkefeld N filter at a negative pressure of 50 cm of mercury. Many experiments were carried out, but only the following will be given since the findings were constant in each instance.

Heat. A group of 9 mice was "hot-room" treated as follows: 6 were inoculated with the virus; the remaining 3 were used as controls. One-half hour after the inoculation, all the animals were placed in the hot room at 37°C and a relative humidity of 100%, for 24 hours. They were transferred to room temperature, 18°C, for 20 hours and then returned to the hot room for 10 hours. This completed the "hot-room" treatment. The animals were sacrificed and autopsied at the end of the fifth day. The data are in Table I.

Cold. A group of 9 mice was "cold treated." Six were given the usual inoculation of virus and the 3 controls were given an equivalent inoculation of nutrient broth. Two hours after the inoculation the mice were thoroughly drenched with cold water and placed in the cold room at 5°C for 5 minutes; they became motionless and cyanosed, and were then placed at room temperature. Two hours later the animals had regained their usual activity. The next day, the process was repeated but the mice were carefully watched and

TABLE I.
Influence of Heat on Resistance of Mice to Influenza Virus.

	Mouse No.	Consolidation of lungs*					Congestion†
		Left	Right				
			Upper	Middle	Lower	Median	
"Hot-Room"	1	+	0	+	+	+	Medium
Treated +	2	+	+	+	+	+	"
Virus, 1:100,000	3	+++	0	+	0	0	Extensive
Dilution	4	+	0	0	+	+	"
	5	++	+	+	+	+	"
	6	+	+	0	0	+	"
Controls	1	+	0	+	+	0	Slight
Virus, 1:100,000	2	0	0	0	0	+++	"
Dilution	3	0	+	0	0	++	Medium
Controls	1	0	0	0	0	0	Slight
"Hot-Room"	2	0	0	0	0	0	"
Treated	3	0	0	0	0	0	"

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = "Blood-shot" lung.

kept in the cold room only until they shivered. They were placed at room temperature and observed over a period of 5 days when they were sacrificed and autopsied. The data are given in Table II.

Fatigue. The usual group consisting of 9 mice was fatigued in a slowly revolving treadmill. This instrument was a circular cage 12

TABLE II.
Influence of Cold on Resistance of Mice to Influenza Virus.

	Mouse No.	Consolidation of lungs*					Congestion†
		Left	Right				
			Upper	Middle	Lower	Median	
"Cold-Treated"	1	0	+	++++	0	0	Slight
Mice + Virus	2	+	0	0	+	0	Medium
1:100,000	3	+	0	0	0	0	Extensive
Dilution	4	+	+	+	+	++	"
	5	++	+	0	+	++	"
	6	+	+	++	0	+	"
Controls	1	+	0	+	+	0	Slight
Virus, 1:100,000	2	0	0	0	0	+++	"
Dilution	3	0	+	0	0	++	Medium
Controls	1	0	+	0	0	+	"
"Cold-Treated"	2	0	0	0	+	0	"
Mice	3	0	0	0	+	0	"

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
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inches in diameter, made of wire and rotated with an electric motor. During a period of 48 hours before inoculation the mice were exercised a total of 30 hours. The rests were not evenly spaced. However, the mice were thoroughly exhausted at the time of inoculation. Twenty of the 48 hours following the inoculation were spent in the treadmill; rests were evenly spaced. The animals were dispatched and autopsied at the end of 5 days. In one of the duplicate studies certain variations were introduced into the experiment; the mice before inoculation were exercised in the treadmill for 60 hours out of a total of 120. The rest periods again were not evenly spaced. The animals were quite exhausted before inoculation. Of the 48 hours following inoculation, 22 were spent in the treadmill; rests were evenly spaced. The data are given in Table III.

Alcohol. Each of a group of 9 mice was injected intraperitoneally with 0.5 cc of a 10% solution of ethyl alcohol. Five minutes after receiving the alcohol, 6 mice were inoculated as usual with the virus. Three mice remained as controls. As a result of the alcohol treatment the mice were locally irritated and staggered about in their cage for approximately 20 minutes. Twenty-four hours later the treatment with alcohol was again repeated. At the end of 5 days the animals were sacrificed and autopsied. The data are presented in Table IV.

Summary and Comment. On reference to Tables I, II, III, and IV, attention is directed to the amount of virus inoculated: 0.05 cc

TABLE III.
Influence of Fatigue on Resistance of Mice to Influenza Virus.

	Mouse No.	Consolidation of lungs*					Congestion†
			Right				
		Left	Upper	Middle	Lower	Median	
Fatigued Mice + Virus, 1:100,000 Dilution	1	+++	+++	0	+	+	Slight
	2	+++	0	+	+	+	Extensive
	3	+	++	0	+	++	Medium
	4	+	++++	0	+	+	"
	5	++	+	0	+	++	Extensive
	6	++	0	+	+	0	"
Controls Virus, 1:100,000 Dilution	1	+	0	+	+	0	Slight
	2	++	0	0	0	+++	"
	3	0	+	0	0	++	Medium
Controls Fatigued Mice	1	0	0	0	0	0	"
	2	0	0	0	0	0	"
	3	0	0	0	0	0	"

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†Slight congestion = pinkness of lung gone.
Medium congestion = red lung.
Extensive congestion = "Blood-shot" lung.

TABLE IV.
Influence of Alcohol on Resistance of Mice to Influenza Virus.

	Mouse No.	Consolidation of lungs*					Congestion†
		Left	Right				
			Upper	Middle	Lower	Median	
"Alcohol-Treated"	1	0	0	0	++	++	Medium
+ Virus, 1:100,000	2	+	0	++	0	++	"
Dilution	3	+	+	+	+	+	Slight
	4	++	+	+	0	0	Extensive
	5	+++	+	+	0	+	"
	6	+	++	+	+	+	"
Controls	1	+	0	+	+	0	Slight
Virus, 1:100,000	2	0	0	0	0	+++	"
Dilution	3	0	+	0	0	++	Medium
Controls	1	0	0	0	0	0	Slight
"Alcohol-Treated"	2	0	0	0	0	0	"
	3	0	0	0	0	0	Medium

*+ to ++++ = degree of lung consolidation.
†Slight congestion = pinkness of lung gone.
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of 1:100,000 dilution is a sublethal dose. From the results, it is evident that these 4 methods did not materially lower the resistance of the mice to the virus of influenza. Furthermore, fatiguing the group of mice in the one experiment about twice as long as those in the other failed to reveal any difference in the amount of lung consolidation between the two experiments.

This leads to an interesting speculation in the interpretation of the data. Working with experimental pneumococcus pneumonia in rats, Klepser and Nungester[1] were able to demonstrate a distinct influence of alcohol on the incidence of infection. This is in keeping with the oft quoted statement that alcoholism and pneumonia in human beings go hand in hand. The belief on the part of the laity in associating exposure with pneumonia has considerable basis in fact. Yet pneumococcus pneumonia stands out not as an epidemic disease but as an isolated condition. On the other hand, influenza is a distinct epidemic disease. It sweeps through a community, on the basis of the 1918-19 statistics, and infects individuals in its wake regardless of the general physical condition of the individual. Thus, it would appear from observations recorded during epidemics and supported by the data obtained in these experiments that the appearance of influenza in an individual depends on the amount of virus and virulence of the virus rather than the general resistance of the host.

[1] Klepser, R. G., and Nungester, W. J., *J. Infect. Dis.*, 1939, **65**, 196.