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AN INVESTIGATION ON THE INFLUENCE UPON THE VITAL RESISTANCE OF ANIMALS TO THE MICRO-ORGANISMS OF DISEASE BROUGHT ABOUT BY PROLONGED SOJOURN IN AN IMPURE ATMOSPHERE

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AN INVESTIGATION ON THE INFLUENCE UPON THE VITAL RESISTANCE OF ANIMALS TO THE MICRO-ORGANISMS OF DISEASE BROUGHT ABOUT BY PROLONGED SOJOURN IN AN IMPURE ATMOSPHERE.

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This is a report of an investigation outlined by, and conducted under the supervision of Drs. John S. Billings and S. Weir Mitchell, in which an attempt has been made to determine whether impure atmosphere produces detrimental influence upon the animal organism as shown in greater susceptibility to certain diseases.

Outline of the Investigation Proposed by Dr. Billings.

"The impurities to be tested are carbonic acid in the proportions of 0.5 to 2.0 per cent. by volume; ammonia and carbonate of ammonia in the proportion of 0.1 to 1.0 per cent.; the products of respiration of a series of animals arranged as in the Brown-Séquard experiments; and the gases from offensive putrefying material.

Afterward it may be desirable to test the effects of sulphuretted hydrogen, and of the vapors of certain volatile organic compounds having offensive odors (skatol, indol, mercaptan).

The micro-organisms to be tested are those of anthrax, streptococcus, diphtheria, tuberculosis, and of croupous pneumonia.

The animals to be used are mice, rabbits, guinea-pigs, and later monkeys.

It is desirable that in each set of experiments the effects of high temperatures (80°-95° F.) be compared with those of lower temperatures (50°-60° F.).

The animals to breathe these mixtures are to be placed in glass jars, or bell-jars, and the inhalation of each mixture should continue for at least one week before inoculations are made, and should continue for a week after the inoculations."

The expenses of this investigation were defrayed out of a grant obtained from the Hodgkins Fund in the hands of the Smithsonian Institution.

Unexpected difficulties were encountered all along in conducting the investigation. The problem of maintaining an atmosphere of fairly constant composition, with the relative proportions of the impurities ranging within the prescribed limits, was a difficult one to solve, and in fact could not be attained with the apparatus employed. It was deemed advisable to expose the animals to the impure atmosphere for at least a month before inoculating them, consequently it was found impossible to maintain the atmosphere at the desired point of impurity during the entire experiment. At times the impurities fell below the prescribed limit, and, in turn, the air supply fell, especially during the night, to a point below that at which it would support life, and some or all of the animals were smothered, and the experiment had to be started over again.

In consequence of these difficulties only six experiments have been brought to a conclusion. A number of others were commenced, but failed through the loss of several or all of the animals; these accidents occurring frequently after the animals had been under experiment for several weeks and were nearly ready for the inoculations. Much time was lost in this manner.

Because of the difficulties encountered, and the indefinite character of the results obtained, only two forms of atmospheric impurities were tested—that of the respiratory impurities with animals in bell-jars arranged in series as in the Brown-Séquard experiments, and the effects of ordinary atmospheric air containing 0.5 to 2.0 per cent. by volume of pure carbonic acid gas. Each form of experiment was repeated successfully three times, using different micro-organisms for the inoculations with each of the three sets of experiments.

A. Inoculations with staphylococcus pyogenes aureus.

Experiment I.

Respiratory impurities.—Six rabbits were placed under bell-jars of 37 litres capacity, arranged in series as in the Brown-Séquard experiment. A current of air was maintained through the series of bell-jars by means of a water pump. The experiment was commenced May 19, 1896, and terminated June 29, 1896. The animals were inoculated on June 23 with 1 cc. of a 24-hour old bouillon culture of staphylococcus pyogenes aureus. The details of the experiment are shown in Table I.

TABLE I.

Date, 1896.	Hour.	No. 1, 2200 g.	No. 2, 2085 g.	No. 3, 1890 g.	No. 4, 1350 g.	No. 5. 1820 g.	No. 6, 2010 g.	C. ft. air per h.	% of CO_2.	Remarks.
May 19....	10.00 a. m.				+	+	+			No. 1 is nearest the pump.
" 22....	4.45 p. m.				1805 g.			5.0		No. 4, 5 and 6 smothered. Continued with four animals.
" 23....	5.00 "							5.25	3.65	
" 26....	4.00 "							3.78	3.75	
June 1....	8.30 a. m.							9.5	3.55	New pump.
" 4....	2.30 p. m.							9.7	2.60	
" 8....	9.15 a. m.							9.7	3.33	
" 13....	2.00 p. m.							9.36	2.42	
" 16....	3.00 p. m.							9.83		
" 23....	10.45 a. m.									Animals inoculated with staphy. pyog. aur.
" 24....	8.45 a. m.			+						No. 3 is dead.
" 29....	11.30 "							9.41	2.16	
" 29....	5.15 p. m.									Experiment stopped. Nos. 1, 2, and 4 living.
		1850 g.	1802 g.		1410 g.					Present weight.

The staphylococcus pyogenes aureus was recovered from the site of inoculation, peritoneal fluid, pleural cavity, and spleen of No. 3.

EXPERIMENTS WITH CARBONIC ACID GAS.

The CO_2 experiments were conducted in the following manner: The animals were all placed under a bell-jar of 37 litres capacity, through which a current of air was maintained by means of a blower operated by the force of the laboratory water supply. The pure carbonic acid gas was derived from a large cylinder of compressed gas, and entered the air supply of the bell-jar through a Y-tube connection. The rate of flow of the carbonic acid gas was regulated by means of the stop-cock on the supply tank. By this means a fairly constant supply could be obtained if carefully watched and regulated.

EXPERIMENT II.

Carbonic acid gas.—Four guinea-pigs were placed under a 37 litre bell-jar. The experiment was commenced May 19, 1896, and terminated June 29, 1896. The details of the experiment are shown in Table II.

TABLE II.

Date, 1896.	Hour.	No 1, 405 g.	No. 2. 315 g.	No. 3, 335 g.	No. 4, 330 g.	% of CO_2.	Remarks.
May 19....	1.00 p. m.						
" 19....	4.00 p. m.					2.21	Air analysis—on air as it enters the bell-jar.
" 20....	9.15 a. m.					8.13	Air analysis—on air as it enters the bell-jar.
" 23....	9.30 "					0.66	Air of bell-jar.
" 26....	4.00 p. m.					0.66	" " " "
June 1....	8.30 a. m.					0.66	" " " "
" 4....	2.30 p. m.					1.80	" " " "
" 8....	9.15 a. m.					0.20	" " " "
" 13....	2.00 p. m.					1.98	" " " "
" 19....	9.00 a. m.			+			No. 3 dead.
" 23....	10.45 "	275 g.	272 g.		265 g.		Present weight.
" 23....							Inoculated with staphy. pyog. aur.
" 24....	8.45 "				+		No. 4 dead.
" 24....	7.30 p. m.		+				No. 2 dead.
" 29....	11.30 a. m.					3.44	Exp. terminated.
							No. 1 seems all right, weight 247 g.

Staphylococcus pyogenes aureus recovered from the site of inoculation, blood, liver, spleen and the peritoneal fluid of Nos. 2 and 4.

Control animals inoculated with staphylococcus pyogenes aureus at the time of inoculating the animals of experiments I and II.

June 23, 1896.

Control rabbit No. 1, weight 1820 g.
" " " 2, " 1410 g.
" " " 3, " 1460 g.
" " " 4, " 1385 g.

Each of these animals was inoculated with 2 cc. of a 24-hour old bouillon culture of staphylococcus pyogenes aureus.

June 23, 1896.

Control guinea-pig No. 1, weight 300 g.
" " " 2, " 420 g.
" " " 3, " 340 g.
" " " 4, " 310 g.

Each of these animals was inoculated with 2 cc. of a 24-hour old bouillon culture of staphylococcus pyogenes aureus.

6/24/96. Exp. I. Rabbit No. 3 died.
6/25/96. Control " " 1 "
6/25/96. " " " 4 "
7/7/96. " guinea-pig " 1 "

All the other animals under experiment and those used as controls are alive 6/29/96.

B. Inoculations with bacillus diphtheriae.

Experiment III.

Brown-Séquard experiment.—Five guinea-pigs were placed in bell-jars of 14 litres capacity arranged in series. Experiment commenced November 7, 1896, and terminated December 19, 1896, when each of the animals was inoculated with 1 mg. of a 24-hour old blood serum culture of bacillus diphtheriae (attenuated). For the details of the experiment see Table III.

Table III.

Date, 1896.	Hour.	No. 1, 640 g.	No. 2, 480 g.	No. 3, 620 g.	No. 4. 590 g.	No. 5, 650 g.	C.ft. air p'rh	% of CO_2.	Remarks.
Nov. 7....	5.00 p. m.							1st bell jar.	No. 1 is nearest the pump.
" 8....	11.30 a. m.						11.0		
" 27....	5.00 p. m.			+	+			4.06	
" 29....	12.00 "			570 g.	595 g.				Nos. 3 and 4 dead. Replaced by fresh pigs.
Dec. 1.....	3.00 "	+						4.74	
" 9....	9.00 a. m.	730 g.							No. 1 dead. Replaced by fresh pig.
" 19....	4.15 p. m.							1.78	Experiment stopped.
" 19....	4.15 "								Inoculated with B. diphtheriae.
		635 g.	430 g.	535 g.	530 g.	525 g.			Present weight.

No. 1 was found dead after 48 hours.
" 2 " " " " 39 "
" 3 " " " " 40 "
" 4 " " " " 39 "
" 5 " " " " 47 "

Experiment IV.

Carbonic acid gas.—Four guinea-pigs were placed under a 37 litre bell-jar, November 17, 1896, and the experiment terminated December 19, 1896, when they were each inoculated with 1 mg. of a 24-hour old blood serum culture of bacillus diphtheriae. The details of the experiment are shown in Table IV.

TABLE IV.

Date, 1896.	Hour.	No. 1, 515 g.	No. 2, 500 g.	No. 3, 525 g.	No. 4, 470 g.	% of CO_2.	Remarks.
Nov. 17 ...	10.30 a. m.						
" 27 ...	9.00 "					2.49	Air entering bell-jar.
Dec. 1....	3.00 p. m.					0.519	" " " "
" 19....	4.00 "					0.67	" " " "
" 19....	4.30 "						Experiment stopped.
" 19....							Inoculated with B. diphtheriae.
		435 g.	400 g.	420 g.	370 g.		Present weight.

No. 1 was found dead after 39 hours.
" 2 " " " " 39 "
" 3 " " " " 39 "
" 4 " " " " 68 "

Control animals inoculated with bacillus diphtheriae.

12/14/96. Control guinea-pig No. 1. Inoculated with 2 mg. of bacillus diphtheriae; died in 4 days.

12/17/96. Control guinea-pig No. 2. Inoculated with 5 mg. of bacillus diphtheriae; died in 2 days.

The animals of experiment III died as follows:

No. 1 was found dead after 48 hours.
" 2 " " " " 39 "
" 3 " " " " 40 "
" 4 " " " " 39 "
" 5 " " " " 47 "

The animals of experiment IV died as follows:

No. 1 was found dead at 39 hours.
" 2 " " " " 39 "
" 3 " " " " 39 "
" 4 " " " " 68 "

The post mortem lesions in all these animals were typical, and the bacillus diphtheriae was recovered from the site of inoculation in each instance.

C. Inoculations with anthrax vaccine, followed by bacillus tuberculosis.

EXPERIMENT V.

Brown-Séquard experiment.—Five guinea-pigs were placed in bell-jars of 14 litres capacity arranged in series. The experiment was commenced February 4, 1897, and terminated February 24, 1897, when the

animals were inoculated with Prof. Chester's First Anthrax Vaccine. The details of the experiment are shown in Table V. As none of the animals were affected by the anthrax vaccine they were inoculated with bacillus tuberculosis, March 4, 1897.

TABLE V.

Date, 1897.	Hour.	No. 1, 322 g.	No. 2, 338 g.	No. 3, 293 g.	No. 4, 318 g.	No. 5, 360 g.	% of CO_2.	Remarks.
eb. 4....	10.30 a. m.							No. 1 is nearest the pump.
" 9....	3.00 p. m.					1.47		Air of bell-jar No. 1.
" 13....	5.00 "					1.46		" " "
" 23....	3.30 "					1.20		" " "
" 24....	4.00 "							Experiment stopped.
" 24....								Inoculated with anthr. vac.
" 24....		298 g.	297 g.	256 g.	286 g.	317 g.		Present weight.
ar. 4								Inoculated with B. tuberculosis.
" 30					+			No. 4 dead. T. bacilli found.
pril 4....				+				" 3 " " "
" 9....						+		" 5 " " "
" 11....		+						" 1 " " "
" 12....			+					" 2 " " "

Tubercle bacilli were demonstrated in the spleen, lymphatic glands and the lungs of all the animals.

EXPERIMENT VI.

Carbonic acid gas.—Four guinea-pigs were placed under a 37 litre bell-jar, December 28, 1896, and the experiment was terminated February 24, 1897, when they were inoculated with the anthrax vaccine. On March 4, 1897, they were each inoculated with bacillus tuberculosis. For details of the experiment see Table VI.

Tubercle bacilli were demonstrated in the lymphatic glands, lungs and spleen of all the animals.

Control animals of anthrax vaccine and tuberculosis inoculations.

2/24/97. Control guinea-pig No. 1, weight 650 g.

2/24/97. " " " 2, " 750 g.

Inoculated with anthrax vaccine, but failed to die, and were again inoculated with bacillus tuberculosis 3/4/97.

No. 2 died 3/27. Found tubercle bacilli in the spleen and at the site of inoculation.

No. 1 died 4/27. Glands tubercular, also lungs, liver and spleen.

3/4/97. Control guinea-pig No. 3, weight 700 g.

3/4/97. " " " 4, " 700 g.

Inoculated with bacillus tuberculosis.

5/17/97 No. 3 dead. Lungs and glands show masses of tubercules. Liver and spleen smaller numbers.

5/28/97 No. 4 killed. Lungs and glands show masses of tubercles. Liver and spleen smaller numbers, but are very much congested.

TABLE VI.

Date, 1896.	Hour.	No. 1, 200 g.	No. 2, 230 g.	No. 3, 217 g.	No. 4, 195 g.	% of CO_2.	Remarks.
Dec. 28....	3.30 p. m.						
1897.							
Jan. 15....	9.00 a. m.				+		No. 4 dead.
" 18....	3.30 p. m.	+	+			3.47	Air entering bell-jar.
" 25....	10.00 a. m.	272 g.	277 g.		360 g.		Nos. 1 and 2 dead. Replaced by fresh pigs.
" 29....	1.30 p. m.					14.9	Air entering bell-jar.
Feb. 4.....	2.30 "					0.4	" " " ".
" 9.....	3.00 "					0.32	" " " "
" 23.....	3.30 "					7.01	" " " "
" 24.....	3.00 "						Experiment stopped.
" 24.....							Inoculated with anthrax vac.
" 24.....		252 g.	236 g.	248 g.	285 g.		Present weight.
" 25.....			+				No. 2 dead. Examination post mortem negative.
Mar. 4.....							Inoculated with B. tuberculosis.
" 19.....					+		No. 4 dead, bacilli found.
" 30.....				+			" 3 " " "
April 10...		+					" 1 " " "

SUMMARY OF RESULTS.

In the staphylococcus and diphtheria inoculations the cultures used appear to have been insufficiently attenuated to show any difference in the effects produced upon the animals under experiment and the control animals. It is, however, very doubtful whether cultures of these organisms could be attenuated to such a degree as to still kill a weakened animal and not kill a control, healthy animal.

The anthrax vaccines used do not kill a healthy guinea-pig, but it was expected that the animals might present sufficient lowering of the vitality to become affected by the vaccines. This, however, was not the case. The animals having failed to die from the effects of the anthrax vaccines, they were then inoculated with an attenuated culture of tuberculosis. All the animals under experiment died much earlier than the control animals. These results indicate a lowered vitality. Whether this lowered vitality was brought about by the atmospheric conditions under which they had lived, or whether it was brought about solely through changes in their diet while under experiment, or whether both these causes were active in producing the result, it is impossible to say. The animals lost flesh and decreased in weight while under experiment. It is not improbable that the loss in weight and the decrease in vitality are both traceable to the same causes.