LONGEVITY OF B. TUBERCULOSIS IN SPUTUM*

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A Preliminary Communication.

Authorities on tuberculosis are practically unanimous in ascribing the greater part of the spread of this disease to infection with organisms from the sputum of individuals suffering from pulmonary tuberculosis.

Comparatively little work has been done to determine how long tubercle bacilli will retain vitality and remain virulent in the sputum under varying conditions.

Dr. Anna I. Von Sholley reports a series of experiments on 36 pigs†. Dr. John Weinzirl gives an interesting report on work with cultures alone‡.

Acting under the suggestion of Dr. Durgin, and with the authority of the Boston Board of Health, this laboratory began a series of experiments June 25th, 1907.

That these experiments might approximate as closely as possible actual conditions, four rooms were obtained in the tenement house districts; No. 1, dry and sunny; No. 2, dry, well-lighted, but with no sunshine, the exposure being to the north; No. 3, dark and dry (lighted by window from air-shaft and window between this and No. 2; No. 4, dark and damp (a basement).

*Read before the Laboratory Section of the American Public Health Association at Winnipeg, August, 1908.

†Report of the Health Department of the City of New York, 1905, Vol. 2, p. 683.

‡Transactions A. P. H. A., Vol. 32, Part 2, p. 128.

These rooms had, of course, to be kept locked, but screened windows were left open in order to obtain circulation of air.

Owing to the difficulty of procuring suitable rooms but two were ready June 25th, Nos. 2 and 3, experiments being started in Nos. 1 and 4 on July 9th.

Technique. Pieces of wooden tongue depressors (representing wood-work) and woolen carpet (representing carpet or rug) about 1½ by ½ inches were immersed over night in a jar of mixed tubercular sputum obtained from one of the city institutions, a separate sample being obtained for rooms 1 and 4. Controls on both were positive.

The pieces of carpet were simply laid on the floors of the rooms but the pieces of wood were held up by string supports so that both sides might be exposed. The samples were collected in sterile test tubes, care being taken to flame the collecting forceps in alcohol that no tubercle bacilli might be carried from one sample to another.

On arrival of the sample at the laboratory about 3 c. c. of sterile water was added to it, and it was allowed to stand 4 to 5 hours. It was then thoroughly rubbed up, and about 1 c. c. of the suspension thus obtained injected subcutaneously into a guinea pig, all tests being made by animal inoculations.

No test was considered positive unless (a) organisms were demonstrated from the lesions or (b) typical pathological lesions were demonstrated microscopically. (In a few instances, usually occurring when the pig died having been inoculated but three or four weeks, it was necessary to make sections from the liver or the spleen.)

First Experiments.

Collections and inoculations were made each Tuesday and Friday until August 23rd. No positives were obtained from Rooms 1 and 4. Two positives on wood were obtained from

Room 2, three and ten days after seeding. Two on wood from Room 3 after 10 days' and 14 days' exposure.

Owing to the fact that no positives were obtained from Rooms 1 and 4, it was decided to repeat the tests, using the same sputum in all the rooms, but as the day when the rooms were seeded was cloudy, another sputum was obtained for use in the sunshine experiments in Room 1. Mixed sputum was used as in the previous experiments.

Second Experiments.

Rooms 2, 3 and 4 were seeded September 6th, and collections made on the 10th, 13th, 16th, daily to the 28th, 30th, October 2d and 4th. Four positives were obtained from Room 2, all being on carpet, after 12, 21, 24 and 28 days' exposure.

Three positives from Room 3, one on carpet after 17 days, two on wood after 10 and 19 days.

Four positives from Room 4, three on carpet after 4, 13 and 17 days; one on wood after 4 days.

Room 1 (sunlight experiments) was seeded September 26th, and collections made hourly for the first day and then discontinued on account of rain. Six collections from each were made, nine being positive and three unsatisfactory, positives being obtained up to the end of the six hours' exposure.

Owing to the difficulty of conducting the experiments, the rooms being located about two miles from the laboratory and at some distance from each other, and to the inconsistency of the results obtained, many negatives being followed by a positive in several instances, it was decided to run a new set of experiments in the laboratory itself where the varying factors could be more directly under our control.

RESULTS OF FIRST SET OF T. B. EXPERIMENTS

Date	Room 1 Carpet	Room 1 Stick	Room 2 Carpet	Room 2 Stick	Room 3 Carpet	Room 3 Stick	Room 4 Carpet	Room 4 Stick	Totals Pos	Neg	Uns	Total
June 25			Seeded		Seeded							
June 28			—	+	uns.	uns.			1	1	2	4
July 2			—	uns.	—	—				3	1	4
July 5			—	+	—	+			2	2		4
July 9	Seeded		—	—	—	+	Seeded		1	3		4
July 12	—	—	—	uns.	—	—	uns.	—		6	2	8
July 16	—	—	—	uns.	—	—	uns.	—		6	2	8
July 19	—	—	—	—	—	—	—	—		8		8
July 23	—	—	uns.	uns.	—	uns.	—	—		5	3	8
July 26	—	—	—	uns.	—	—	—	—		7	1	8
July 30	—	—	—	—	—	—	—	—		8		8
Aug. 2	—	—	—	—	—	—	—	—		8		8
Aug. 6	—	—	—	—	—	—	—	—		8		8
Aug. 9	—	—	—	—	—	uns.	—	—		7	1	8
Aug. 13	—	—	—	—	—	—	—	—		8		8
Aug. 16	—	—	—	—	—	—	—	—		8		8
Aug. 20	—	—	—	uns.	—	—	—	—		7	1	8
Aug. 23	—	—	—	—	uns.	—	—	—		7	1	8

RESULTS OF SECOND SET OF T. B. EXPERIMENTS

Date of col.	Room 1 Carpet	Room 1 Wood	Room 2 Carpet	Room 2 Wood	Room 3 Carpet	Room 3 Wood	Room 4 Carpet	Room 4 Wood	Pos.	Neg.	Uns.	Tl.
Sept. 10			—	—	—	—	+	+	2	4		6
" 13			—	—	—	—	Uns.	—		5	1	6
" 16			—	—	—	+	Uns.	—	1	4	1	6
" 17			—	—	—	Uns.	—	—		5	1	6
" 18			Uns.	—	—	—	—	—		5	1	6
" 19			—	—	—	—	+	—	1	5		6
" 20			—	—	—	—	—	—		6		6
" 21			—	—	—	uns.	—	—		5	1	6
" 22			—	—	—	—	—	—		6		6
" 23			—	—	+	—	+	—	2	4		6
" 24			—	—	—	—	—	—		6		6
" 25			—	—	—	+	—	—	1	5		6
" 26	(4+ (2 uns)	(5+ (1 uns)	—	—	—	—	—	—	9	6	3	18
" 27			Uns.	—	—	Uns.	—	—		4	2	6
" 28			—	—	—	—	Uns.	—		5	1	6
" 30			Uns.	—	—	—	Uns.	—		4	2	6
Oct. 2			—	—	—	—	—	—		6		6
Oct. 4			Uns.	—	—	—	—	—		5	1	6
									16	90	14	120

The following modifications of technique were used:

1. Sputum obtained from a single individual was used, control inoculations being made long enough in advance of the inoculations to show that we were dealing with a virulent organism.

2. Sputum was thoroughly shaken to obtain uniformity, and a large drop of the mixture thus obtained was placed on each piece of carpet or stick used.

This gives more nearly the condition of sputum as expectorated, although, of course, the amount used was much smaller.

Third Experiments.

For control, on these experiments two pigs were inoculated December 9, 1907, with sputum from a single patient. One of these controls developed so rapidly that it was possible to demonstrate typical organisms from the inoculation site and inguinal glands by December 20; the other autopsied at a later date was also positive.

On December 23 pieces of carpet and stick were seeded with a second sample from this patient.

Part of these were exposed in a box with a glass front and a cloth back on a high bench behind the microscope bench, the front of the box facing the north windows. These specimens thus obtained abundant diffuse light, but no sunlight, conditions corresponding to Room 2 in the previous experiments.

Others were exposed to the air in a box in a dark stock room, all artificial light excluded by a cloth hung over the front, tacked to the top and sides, conditions corresponding to Room 3. Pigs were inoculated December 26th, and every other day thereafter as long as the material exposed lasted.

From the specimens exposed to light and air the following results were obtained:

Carpet, 8 positives, covering 19 days' exposure, one negative being obtained after 15 days, and all being negative after 19 days; tests covered 57 days.

Wood, 12 positives, covering 31 days' exposure. During this time there were two negatives after 25 and 29 days and one unsatisfactory after 21 days. No further positives were obtained, although the tests covered a period of 70 days.

From the specimens exposed to the air, but kept in the dark, results were as follows:

Carpet, 17 positives. The first 16 tests made were positive, covering 35 days' exposure. No positives were obtained in the next thirty days, when as a result of "rubbing up" two specimens together and making an inoculation one positive was obtained. Although this was repeated for the remaining three inoculations, there were no more positives. The tests covered a period of 70 days.

Wood, 42 positives. One negative was obtained after 71 days' exposure, and there was one unsatisfactory test after 39 days. No end point was reached, positive results being obtained up to 88 days. These tests are being repeated.

One hundred and ten pigs are also now under observation in a series of sunshine experiments using sputum deposited on brick as well as wood and carpet, these specimens being exposed to the direct sunlight on the roof of the building in which the laboratory is situated, hourly inoculations being made from 8 A. M. to 6 P. M. Sputum has for some time been exposed and inoculations will soon be made in tests approximating Room 4 (dark and damp).

Subsequent Tests.

Brick, first day's exposure, 9+ from 12 inoculations.
Brick, second day's exposure, 8+ from 18 inoculations.
Brick, latest + after 31 hours' exposure to weather.
Stick, first day's exposure, 3+ from 8 inoculations.
Stick, latest + after 5 hours' exposure.
Carpet, 2 positives, after 1 and 7 hours' exposure.

Although by no means ready to draw final conclusions from this work the results of the experiments so far concluded indicate:

1. In order to obtain consistent results in experiments of this kind (a) sputum from a single individual which has been tested and found to be virulent should be used; (b) the sputum should be thoroughly mixed by agitation in order that any particle may be a fair sample of the whole; (c) all the conditions should be under the control of the investigator, weather necessarily being excepted.

2. Tubercle bacilli in sputum deposited on carpet will die out much more rapidly than when deposited on wood.

3. As on wood in the dark and dry tests virulent bacilli persisted for three months, and end point not being reached, it is evident that sputum of tubercular patients cannot be too carefully cared for.

4. It is also apparent that the so-called "tubercular houses" are a real menace to the health of a community.

Date	Approx No. 2 Carpet	Approx No. 2 Wood	Approx No. 3 Carpet	Approx No. 3 Wood	Pos.	Neg.	Unsat	Totals
Dec. 26	+	+	+	+	4			4
" 28	+	+	+	+	4			4
" 30	+	+	+	+	4			4
Jan. 1	+	+	+	+	4			4
" 3	+	+	+	+	4			4
" 5	+	+	+	+	4			4
" 7	−	+	+	+	3	1		4
" 9	+	+	+	+	4			4
" 11	+	+	+	+	4			4
" 13	−	uns.	+	+	2	1	1	4
" 15	−	+	+	+	3	1		4
" 17	−	−	+	+	2	2		4
" 19	−	+	+	+	3	1		4
" 21	−	−	+	+	2	2		4
" 23	−	+	+	+	3	1		4
" 25	−	−	+	+	2	2		4
" 27	−	−	−	+	1	3		4
" 29	−	−	−	uns.		3	1	4
" 31	−	−	−	+	1	3		4
" 2	−	−	−	+	1	3		4
" 4	−	−	−	+	1	3		4
" 6	−	−	−	+	1	3		4
" 8	−	−	−	+	1	3		4
" 10	−	−	uns.	+	1	2	1	4
" 12	−	uns.	uns.	+	1	1	2	4
" 14	−	−	−	+	1	3		4
" 16	−	−	−	+	1	3		4
" 18	−	−	−	+	1	3		4
" 20		−	−	+	1	2		4
" 22		−	−	+	1	2		3
" 24		−	−	+	1	2		3
" 26		uns.	+	+	2		1	3
" 28		−	−	+	1	2		3
Mar. 1		−	−	−		3		3
" 3		−	−	+	1	2		3
" 5				+	1			1
" 7				+	1			1
" 9				+	1			1
" 11				+	1			1
" 13				+	1			1
" 16				+	1			1
" 18				+	1			1
Totals	28	35	35	42	77	57	6	140

The following papers read at the meeting of the Laboratory Section at Winnipeg were not received for publication:

"Experimental Leucocytosis in the Cow's Udder," Conrad Hoffman.

"The Individual Animal as a Factor in the Germ Control of Milk Supplies," E. G. Hastings and Conrad Hoffman.

"The Waters of the Great Lakes," R. B. Dole.

"Some Studies of the Physiological Leocyte Content of Cow's Milk," B. H. Stone and L. P. Sprague (Journal of Medical Research, 1909).

"A Comparison of Practical Methods for Determining the Bacterial Content of Milk," P. G. Heineman (Journal of Infectious Diseases, Vol. V., p. 412).

"Stability and Putrescibility in Sewage Filter Effluents," E. B. Phelps.

"Tests for the Significance of Gas-Producing Bacteria in Milk," D. D. Jackson and H. W. Streter.