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According to Fraentzel and Runkwitz,<sup>5</sup> Amann,<sup>1</sup> and more recently Feigel<sup>4</sup> and Immerwahr,<sup>6</sup> as well as other observers, microscopical changes which they consider indicative of degeneration are observable in the tubercle bacilli contained in the sputum of tuberculous cases undergoing the Koch treatment. It seemed advisable on this account to test the virulence of the bacilli in such cases by inoculations on animals,—for if these changes in the bacilli are really degenerative, then we may reasonably expect that the bacilli will show signs of attenuation by their affecting inoculated animals but slightly or not at all.

Guinea-pigs were chosen for these experiments because of the fact that they give more regular results than rabbits. Of 33 animals inoculated with sputum 15 died of septicaemia within the first few days after inoculation—the loss of many animals from this cause being an unavoidable part of such sputum inoculations. The following notes relate to 18 guinea-pigs, 5 of which were inoculated by way of control with the sputum of a patient who was not undergoing the Koch treatment—the 13 remaining animals having been inoculated with the sputum of different cases undergoing the treatment.

A brief outline of the history of each case<sup>a</sup> from which sputum was taken for the inoculations on the 13 last-named guinea-pigs is appended to the accompanying tables. The relative number of tubercle bacilli in the various sputa used is roughly indicated by the signs \* (many), \*\* (very many), \*\*\* (enormous number), placed under the amount of sputum inoculated, noted in the third

<sup>a</sup> Abstracted from the Clinical Records of the Johns Hopkins Hospital.



column. The sputum with which guinea-pig No. 13 was inoculated contained but few bacilli. Guinea-pigs Nos. 10-11 were inoculated hypodermically with a salt solution suspension of small cheesy almost spherical masses measuring respectively  $\frac{1}{4}$  and  $\frac{1}{2}$  cm. across—these masses were almost pure cultures of very small beaded tubercle bacilli and represented an enormous number of these organisms. The cheesy masses were broken up in a small sterilized mortar and pestle (made of glass tubing) into which broken glass had been introduced. It was found that the most satisfactory way of inoculating guinea-pigs with sputum was to inject the latter hypodermically with a large syringe.

### CASE I.

William P. L.—, aet. 50, weight 114½ lbs., single. Machinist on ships. Admitted to Hospital December 11, 1890. Began to cough 9 months ago at which time he weighed 145 lbs. Cough not severe, expectoration scanty, mucoid. No haemoptysis. Sputum examined 6 months ago contained tubercle bacilli but no elastic tissue. Four months ago his weight had fallen to 106 lbs. On treatment at the time the patient's condition improved. On admittance the patient appears but slightly emaciated, looks well. Temperature 99-100° F. Chest fairly broad and quite deep. Total expansion 5 cm. Percussion on the right side over first rib and second interspace and third rib gives high pitched resonance—not definite dulness. Slightly defective resonance in clavicular and supra-clavicular region. Rest of lung clear. On the left side slightly impaired resonance in supra-spinous fossa, lung clear below. In inter-scapular region on both sides resonance a little skodaic. Fremitus a little more marked in the left than right mammary region. Behind, fremitus a little more marked on left than on right side.

On auscultation, breath sounds nowhere intense. At right apex at end of inspiration a few crackling râles. At left apex both on in- and expiration distinctly cavernous and on coughing crackling râles heard. No other appreciable alterations in front, except that expiration is everywhere a little prolonged. Behind at right apex rather deep broncho-vesicular breathing, fine crackling râles at end of inspiration, toward base breath-sounds become louder. On the left side breathing at apex approaches cavernous, a few râles on coughing—breath sounds loud and clear at base. Expiration on both sides distinctly prolonged.

Other organs normal. Patient improved under Koch treatment, coughed less, expectoration ceased, weight rose 3 lbs. On the 8th of January, fewer râles, the condition of lungs otherwise about the same. Patient left Hospital February 26, 1891.

### CASE II.

K—, aet. 29, weight 99 lbs., married. Admitted to the Hospital December 15, 1890. Cough began in the autumn of 1888, with loss of flesh and bacilli in the sputum. In January, 1890, patient had influenza after

I.

No. of Experiment.	Date of inoculation of animal with sputum.	Amount of sputum inoculated.	No. and dose of tuberculin inoculations received by patient on or before the date given.	Weight of animal (in grammes) at time of inoculation and at death or shortly before death.	No. of days animal lived after inoculation.	Post-mortem Notes. Including microscopical examination of tissues by means of sections and smear-preparations.	Positive or negative result expressed by signs.
1	Dec. 21.	1.5 cc.	.....	556-315	46	Seat of inoculation ulcerated. Inguinal and mesenteric lymph-glands enlarged. Liver and spleen tuberculous, sanguineous fluid in peritoneum. Lungs pneumonic. Tubercle bacilli found in liver, spleen and lymph-glands.	+
2	Dec. 21.	1.5 cc.	.....	545-385	50	Inguinal and mesenteric glands enlarged and caseous. Liver, spleen and lungs presented tuberculous nodules. Tubercle bacilli found in all the organs mentioned.	+
3	Dec. 21.	1.5 cc.	.....	648-420	26	Lymphatic glands generally enlarged. Left axillary gland enormously enlarged forming pus-sack. Spleen tuberculous. One tubercle on edge of lung. Bacilli found in lymph glands and spleen.	+
4	Dec. 21.	1.5 cc.	.....	437-265	25	Found dead, its abdomen eaten open by other guinea-pigs. Animal much emaciated. Lymphatic glands generally tuberculous—one axillary gland as in preceding case. Bacilli found in glands.	+
5	Dec. 21.	1.5 cc.	.....	289-204	26	Lymphatic glands generally tuberculous. Miliary tubercles in lung and spleen—bacilli found.	+

II.

No. of Experiment.	Date of inoculation of animal with sputum.	Amount of sputum inoculated (in cubic centimeters).	No. and dose of tuberculin inoculations received by patient on or before the date given.	Weight of animal (in grammes) at time of inoculation and at death or shortly before death.	No. of days animal lived after inoculation.	Post-mortem Notes. Including microscopical examination of tissues by means of sections and smear-preparations.	Positive or negative result expressed by signs.
1	Jan. 4.	1.5 *	8 inoc's, 4 mgr. (Case II)	417-280	18	No signs whatever of tuberculosis, no bacilli found anywhere. Died from chronic septicaemia.	-
2	Dec. 21.	1.5 **	9 inoc's, 8 mgr. (Case L)	515-300	59	Cheesy mass at seat of inoculation containing tubercle bacilli. Miliary tubercles in lung, liver and spleen. Lymphatic glands caseous—tubercle bacilli found in the tissues named.	+
3	Dec. 21.	1.5 **	9 inoc's, 8 mgr. (Case L)	173-205	34	Hemorrhage from nose. Seat of inoculation ulcerated. Inguinal, bronchial and mesenteric lymph-glands enlarged, tuberculous. Clot in trachea. Miliary tubercles found in lung, liver and spleen—tubercle bacilli found in latter and in lymph glands.	+
4	Dec. 21.	1.5 **	9 inoc's, 8 mgr. (Case L)	497-312	64	Inguinal and bronchial glands much enlarged, caseous. Tubercles in liver, spleen and lungs, bacilli being found therein as also in the caseous glands. Intestine congested in places.	+
5	Jan. 29.	3.	15 inoc's, 11 mgr. (Case III)	275----	17	Seat of inoculation ulcerated. Lymph-glands enlarged and caseous. Lungs congested, contain small tuberculous nodules. A few small tubercles in the liver. Spleen apparently normal. Bacilli found in lungs, liver and lymph-glands.	+
6	Jan. 16.	2 *	16 inoc's, 6 mgr. (Case II)	440-280	23	Indurated ulcer at seat of inoculation. Inguinal glands enlarged, caseous. Miliary tubercles in lung, spleen and liver. Tubercle bacilli found in caseous glands, lung, liver and spleen.	+
7	Jan. 16	2.5 *	16 inoc's, 6 mgr. (Case II)	330-205	18	Showed signs of paralysis two days before death. Seat of inoculation ulcerated. Inguinal glands on side inoculated and mesenteric glands enlarged. Lungs pneumonic. Spleen enlarged, tuberculous, highly pigmented. Bacilli found in spleen and glands.	+
8	Feb. 10.	2.75 *	18 inoc's, 11 mgr. (Case V)	575-370	19	Seat of inoculation ulcerated. Lymph-glands enlarged. Liver, lungs and spleen tuberculous, contained bacilli.	+
9	Jan. 8.	2.5 *	20 inoc's, 19 mgr. (Case I)	509-495	22	No signs whatever of tuberculosis. Small necrotic areas in liver. Spleen contained much pigment. Died from chronic septicaemia.	-
10	Jan. 24.	***	21 inoc's, 7 mgr. (Case II)	270-180	36	Seat of inoculation ulcerated. All the lymphatic glands, as also liver, lung and spleen, showed tuberculous tissue and contained tubercle bacilli.	+
11	Jan. 28.	***	23 inoc's, 7 mgr (Case II)	263-210	33	No evidence of tuberculosis. Lymph glands enlarged. Liver, apparently normal. Spleen highly pigmented. Lungs pneumonic.	-
12	Jan. 29.	2. *	27 inoc's, 36 mgr. (Case IV)	250-210	18	Seat of inoculation ulcerated. Lymphatic glands enlarged. Liver, apparently normal. Spleen highly pigmented. Bacilli found in glands.	+
13	Jan. 29.	1.	29 inoc's, 36 mgr. (Case VI)	510-340	17	Large ulceration at seat of inoculation. Lymph glands enlarged. Liver, apparently normal. Spleen pigmented. Lungs pneumonic. Tubercle bacilli found in lymph glands.	+



which she developed signs in the lungs. Had bronchitis and clear evidence of cavity formation at right apex. She improved subsequently. On entering the hospital a cavity at the right apex and some consolidation of left apex observed—the rest of the lungs presenting no abnormal signs. The patient left unimproved, February 16, 1891.

### CASE III.

Robert D—, aet. 34, weight 155 lbs., single, laborer. Admitted to the Hospital December 24, 1890. Began to cough about four months ago. Coughed but little, with occasional appearance of blood. Complains of but slight fevers at night, no sweats. His weight in summer was 170 lbs.—has been working hard. Patient strong and healthy looking. Temperature 101–102.8° F. Chest well formed—circumference 38 inches, expansion  $\frac{3}{4}$  inch. Tactile fremitus slight and equal on both sides. Percussion on right side at apex and over clavicle, gave slightly deficient resonance. In the outer mammary region and extending into extreme upper axilla, slight dulness. Left lung clear in front and behind. Right lung behind at extreme apex clear on percussion, below and to outer side of spine of scapula defective resonance—the rest of the lung clear. Auscultation, on right side in front clear everywhere, in upper axillary region tubular breathing very marked—bronchophony and whispered voice. On coughing a few crepitant râles. Behind, above spine of scapula clear, over the area of dulness above noted—the same signs as in axilla. Other organs normal.

The patient left the Hospital on February 24th much improved in condition, having gained 7 $\frac{3}{4}$  lbs., the cough almost gone, the expectoration reduced to about 2 cc. each 24 hours, and containing but few bacilli.

### CASE IV.

Kate J—, aet. 32, weight 91 lbs., married. Admitted to the Hospital December 12, 1890. Troubled with cough for the last four years during the winter. Hardly any expectoration until the spring of 1890—the sputum thick and once or twice tinged with blood. Very little sweating at night, does not complain of fever. No loss of flesh, no gastric symptoms. The sputum on admission amounted to 20–40 cc. in the 24 hours, contained elastic tissue and many bacilli. Temperature normal. Pulse 80. Respirations quiet. Expansion equal and good on both sides. Tactile fremitus equal and normal. Left clavicle a little more prominent than right—slight flatness on that side. On percussion above clavicle and in infra-clavicular region on the left side, high pitched note extending as far as the third rib. Behind, the note at left apex flatter than on right side. On auscultation on the left side breath sounds observed to be feeble, somewhat tubular particularly on expiration, very few râles heard on quiet inspiration—on deep inspiration many râles of medium size, larger and louder on coughing were heard. Behind, on coughing, râles heard as low as the middle of the scapula. A few râles at apex as well as in inter-scapular region. Right lung clear on percussion, throughout. A few râles in front at right apex.

Patient improved under treatment gaining  $13\frac{3}{4}$  lbs. Left the Hospital April 4, 1891.

#### CASE V.

John H—, aet. 35, weight 132 lbs., married. Wheelwright by occupation. Admitted to the Hospital January 2, 1890. Cough began one year ago after an attack of influenza, but did not become constant until 3–4 months ago. No expectoration or night-sweats till a few weeks ago. Looks healthy and well nourished. Temperature  $98-100^{\circ}$  F. *Examination*: Dulness from third to middle of fifth rib and from left border of sternum to 3 cm. outside nipple. Over this area tubular breathing with sharp liquid râles on expiration and coughing. Bronchophony and pectoriloquy over an area 4 cm. in diameter above and to outside of nipple. Dulness toward axilla somewhat increased after inoculations with Koch remedy. When the patient left at the expiration of two months he coughed less, his general condition was improved his weight being  $136\frac{1}{2}$  lbs.

#### CASE VI.

Kate K—, aet. 30, weight  $111\frac{1}{2}$  lbs., milliner. Admitted to the Hospital December 12, 1890. Complained of slight cough but did not have much fever. On examination, expansion in right infra-clavicular space found defective. Resonance higher in pitch as low as third rib—the breath sounds being feeble. Expiration somewhat tubular. Moist râles large and medium sized as low as fourth rib. Behind, defective resonance in supra-scapular region—moist sounds as low as middle of scapula. Left lung uninvolved. Temperature on admittance  $98.2^{\circ}$  F. Bacilli in sputum in small numbers. No elastic tissue. Up to about the 15th of February, 1891, patient showed improvement—cough moderated, sputum lessened in quantity and weight rose to  $118\frac{1}{2}$  lbs. At time of writing patient is still in the Hospital, expectation has again increased, her condition not improved.

Of the 13 guinea-pigs inoculated with the sputum of patients undergoing the Koch treatment, (see Table II) ten became tuberculous. From this we may conclude that the bacilli in the sputum were still virulent. We have the following grounds for not laying too much weight on the three negative results obtained.

Völsch<sup>11</sup> (1889) observed that the development of tuberculosis was considerably retarded or even checked when rabbit-septicaemia bacilli introduced with tuberculous sputum multiplied in the animal inoculated. Depending upon the number of rabbit-septicaemia organisms in the sputum, as also their rate of development, the septicaemia produced was either chronic or acute—he coincided with Smith<sup>10</sup> (1886) in the latter observation.

In this connection it is important to note that Welch<sup>12</sup> has observed under normal conditions a wide variation in the degree

of virulence of the organism of sputum-septicaemia. Depending upon the grade of virulence of the organism, the septicaemia was rapid or slow, or the animal remained quite unaffected by the inoculation.

This antagonism between two different pathogenic organisms in the body of an animal has also been observed in other cases to lead to recovery or retardation in the progress of a disease.

Emmerich and Mattei<sup>2, 3</sup> showed that a number of animals inoculated with bacillus anthracis did not die as is usual of anthrax if they had been previously or subsequently inoculated with cultures of staphylococcus pyogenes aureus—the introduction of the latter organism in these cases retarded or completely checked the growth of anthrax bacilli in the animal. A large number of anthrax bacilli were completely destroyed in some cases.

Pawlowsky<sup>9</sup> followed out this line of research. He in a number of instances cured localized anthrax by inoculating Friedländer's pneumo-bacillus. Staphylococcus pyogenes aureus and bacillus prodigiosus did not have as much effect. When the anthrax infection was of a general character, inoculation with pneumo-bacillus retarded the progress of the disease and in a few cases a cure was produced.

Kostjurin and Krainski,<sup>8</sup> in a preliminary note entitled "On the action of putrefaction, and tubercle-toxines on animals and their influence on experimental tuberculosis," state that it is highly probable these bodies introduced subcutaneously in an animal retard the further development of tuberculosis and may render an animal immune against renewed infection—besides this, they seem to render the tuberculous process simply a local one and promote caseation.

The observations of the foregoing experimenters show us that it is necessary to be cautious in interpreting our results, particularly when negative ones are in the minority. From the few experiments here given it is difficult to form a positive opinion. We are inclined to conclude that as long as the majority of our animals died with tuberculous lesions the tubercle bacilli were not attenuated. A certain number of the results in such experiments must inevitably be obscure as we are always dealing more or less with unknown quantities when we inoculate an animal with such a septic putrefactive mass as tuberculous sputum. There seems, however, to be no other way out of it than to carefully continue this line of

experimentation on a larger scale *as mere microscopical observation cannot determine the matter*, and we have no other means at hand of settling the question than by direct inoculations of animals.

It will be remembered that Koch<sup>7</sup> in his second communication said, "Only this much is certain, we have not to deal with a killing off of the tubercle bacilli in the tissues—it is the tissue which contains the bacilli which is acted upon by the remedy."

The writers regret to state that these inoculations were made before the method for estimating the actual number of tubercle bacilli in sputum, described in an accompanying article, had been perfected, and consequently as in all similar experiments as yet made, an unknown number of organisms was introduced into the animals with the sputum.

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