the obstructive lesion in the nose. The melancholic cast is the most striking and constant feature of the cases seen by me, as also of cases reported by others. The patient is depressed, disheartened, and lacks both incentive and energy for work; he has forebodings of ills, mistrusts his friends, and wishes to be alone; imagines that no one is interested in him, and that he had as well be dead as living; in some cases, as in one of mine, he seeks to rid himself of his afflictions by suicide.

It has occurred to me that the sigh which is associated always with sadness, grief, or otherwise depressed mental state is but an illustration of what we have here represented. For what is the sigh, physiologically considered, but an extraordinary respiratory effort to compensate for a deficient supply of oxygen to the lungs which must attend a nasal stenosis?

Did Shakespeare think of this when he wrote:

> "There's matter in these sighs, these profound heaves;
> You must translate; tis fit we understand them."

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## EXPERIMENTAL STUDIES ON THE PREPARATION AND EFFECTS OF ANTITOXINS FOR TUBERCULOSIS.

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THE workers in this field have not been idle, though it must be conceded that few definite good results have been attained, and but little progress has as yet rewarded the patient toil expended in attempts to produce a curative or antitoxic serum for tuberculosis.

Simultaneously with the development of tetanus and diphtheria antitoxin—even earlier (1888)—Héricourt and Richet[23] reported favorable results in transferring immunity to tuberculosis from dogs to rabbits by serum injections. Unfortunately, clinical tests of the efficacy of this serum when applied to human tuberculosis did not prove at all encouraging. Nevertheless, the efforts of experimenters to advance existing knowledge of the toxic products of the tubercle bacilli, to produce an artificial immunity against tuberculosis in animals, and to obtain an antitoxic serum for this disease have been steadily carried on, and some light has been thrown on this all-important and complex problem.

One of us (Trudeau[24, 25]) has been engaged in experiments on immunity to tuberculosis since 1891. The present paper, however, includes only

our studies with serums which were commenced in 1894. While confessing our disappointment nt the outcome of most of these experiments, we yet feel warranted in presenting them, because they seem to us to indicate some interesting phases of work in tuberculosis and the care needed to make safe deductioas from laboratory experiments.

By nnalogy the efficacy of any antitoxic serum and the ease with which it is obtainable would seem to be in direct ratio to the degree of toxicity possessed by the poison against which protection is sought. For this reason, to produce and demonstrate antitoxic properties in serums would, *à priori*, be more difficult in n disease so chronic as tuberculosis, on account of the low degree of toxicity of its products. Our attempts to produce serums by our own methods, the antitoxic power of which could be proved experimentally on animals; or to demonstrate antitoxic properties in the serums produced by others, hnve shown only slight potency for any of them up to the present time. Tuberculosis does not belong to that class known as the acute infectious diseases which kill by ncute toxæmia, but to the group known as the infectious granulomata to which syphilis, nctiaomycosis, and leprosy also belong, and which destroy life not only by the chroaic and long-continued systemic poisoaiag they produce, but by the pathological changes brought about through the localization and growth of the germs in organs necessary to life.

Koch's[41] assumption seems plausible, that immunity to the toxio products of tuberculosis does not necessarily imply immunity to tuberculosis, and a serum which would neutralize the toxic effects of tuberculin may not prevent the growth of the tubercle bacillus in the tissues and its destructive actioa on the organs of the body. An efficacious serum for this disease would probably require, therefore, to possess not alone antitoxic, but also germicidal properties; or, nt least, the power to excite the organism to germicidal activity. Bactericidal properties have been claimed for certain serums by careful observers, but the specificity of any such action is open to question nnd the existence of any demonstrable degree of germicidal power in nntituberculous serums has not been confirmed generally by other observers.

It is not nt all certain that even if bacterial immunity could be produced by any method, the serum of nnimals possessing this immunity would necessarily be either antitoxic or germicidal. Indeed, one of us (Trudeau[38]) has succeeded in producing n marked degree of immunity in rabbits by preventive inoculation of living cultures of tubercle bacilli attenuated by prolonged cultivntion; nnd yet the serum of these animals which had resisted a snbsequent virulent inoculation proved to hnve but slight if any antitoxic power, nnd did not seem to influence to nn nppreciable degree the course of the disease in tuberculous guinea-pigs.

Notwithstanding the meagre results obtained in animal experiments, a good deal of clinical evidence as to the value of serum-treatment has been presented, which, however, is not sufficiently encouraging to be convincing in tuberculosis, a disease which runs without any specific treatment so varied and erratic a course. The clinical evidence has not been considered in this research, and must be judged by itself. These studies have been entirely confined to such experimental proof of the presence of curative and antitoxic properties in serums as could be obtained by laboratory methods.

The nature of the bacterial poisons used in injecting animals with a view to producing curative serums is probably of vital importance to the success of such attempts.

When we began this work the observations of Hammerschlag,[32] Koch,[46] Proskauer and Brieger[21], Hueppe and Scholl,[29] Weyl,[35] Héricourt and Richét,[34] Crookshank and Herroun,[22] Richét,[76] Bâbes,[4] Zuelzer,[97] Klebs,[43] Hahn,[36] Kühne,[49] Hoffman,[38] Matthes,[62] de Schweinitz,[82] and our own agreed in the main as to the presence of poisonous albuminous substances in cultures of tubercle bacilli, which were products of the germ-growth and had chemical reactions like the albumoses, albuminates, and nucleo-proteids,* and all producing the characteristic physiologic action of tuberculin. It is to be noted that large quantities of the filtered culture fluid are borne by healthy animals without immediate toxic effects, while quite small doses may produce death in tuberculous animals within a few hours.

No material addition to our knowledge of these culture-products has been published to the present time. Behring[8] has recently reported the separation of a more active poison than hitherto obtained. De Schweinitz and Dorset[83] have prepared small quantities of a necrotizing substance. More recently Hahn[31] obtained from crushed living tubercle bacilli a juice having the properties of a hydrolytic ferment. The tubercle bacilli have been found by de Schweinitz,[81] Koch and Proskauer,[47] and Unna[91] to contain considerable quantities of fat and cellulose, the former having the specific staining reaction. The whole subject evidently requires exhaustive study.

The experiments with dead tubercle bacilli and their extracts by Wyssokowicz,[96] Maffucci,[54 55] Daremberg,[74] Prudden and Hodenpyl,[73] Koch,[46] Straus and Gamaléïa,[86] Vissman,[94] Kostenitch,[48] Grancher and Martin,[29] Grancher and Ledoux-Lebard,[23] Freudenreich,[27] Masur and Kockel,[62] Abel,[1] Curriére,[71] Sciolla,[80] and Bâhes and Proca,[7] show their marked locally irritant character, and their power to produce tubercles,

* A single phosphorus determination, kindly made for us by Prof. R. H. Chittenden, of the pure proteid obtained from cultures on synthetic media (containing no peptones nor albumi-noid) gave a content of 1.52 per cent. This indicated the presence of considerable nucleo-proteid produced by the tubercle bacilli.

aseptic abscesses, cachexia with grave disturbance of the blood-forming functions, and nephritis. In the face of these facts it seemed hardly probable that it would be practicable to use cultures containing tubercle bacilli, living or dead, to create and increase tolerance. At least it appeared unlikely that animals would tolerate doses presumed to be necessary in order to originate antagonistic substances. That the tubercle bacilli substance is necessary to produce immunity was later claimed by Koch in the experiments with tuberculin "R."

We have endeavored to cover many, but not all, aspects of this subject in our experiments, and our methods have varied in some respects from those of other workers in this field whose developments we have followed. Without further discussing the theoretical considerations concerned, we will proceed to the description of our work.

The studies included in Part I. relate to the methods adopted by us in attempts to produce the sought-for immunity in various animals, and the tests of the germicidal and curative properties which might be possessed by such serums. The studies included in Part II. relate mostly to tests in animals of the antitoxic power of serums in tuberculin poisoning.

Thus far we have employed sheep, fowls, asses, and rabbits in attempts to obtain antitoxic serums. Before giving details of the work the following summary will set forth the general methods employed by us. We obtained serums from:

I. Sheep repeatedly inoculated intravenously with filtrate of cultures of tubercle bacilli on thymus bouillon.

II. Fowls that were repeatedly inoculated intraperitoneally with tubercle bacilli of mammalian tuberculosis of increasing virulence, and recovered.

III. Sheep injected subcutaneously with increasing doses of tuberculin.*

IV. Sheep repeatedly inoculated intravenously with living non-virulent cultures of tubercle bacilli.†

V. Ass repeatedly inoculated intravenously with living, non-virulent cultures of tubercle bacilli.

VI. Ass inoculated: (*a*) Subcutaneously with virulent living cultures of tubercle bacilli; (*b*) intravenously with virulent tuberculous material, and recovered; (*c*) treated with tuberculin subcutaneously in increasing doses.

VII. Ass injected: (*a*) Subcutaneously with dead cultures of non

* The tuberculin used in this work was made from full-grown bouillon cultures of non-virulent tubercle bacilli from human source, evaporated over a water-bath to one-tenth volume and filtered through clay. 0.100 c.c. usually sufficed to kill guinea-pigs six weeks tuberculous.

† The cultures denoted "non-virulent" were from tubercle bacilli of human origin, grown four years in the incubator, and which only occasionally killed guinea-pigs in six months to one year.

viruleat tuhercle bacilli on thymus houillon; (*b*) with precipitated tuberculin from cultures of tbe same non-virulent tubercle bacilli on proteid-free media; (*c*) nlkaliae extracts of the bacilli with dead bacilli, suhcutaneously; (*d*) living non-virulent tuhercle bacilli.

VIII. Rahbits: (*a*) Inoculated intravenously with non-virulent tuhercle hacilli nnd recovered; (*b*) inoculated intraperitoneally with virulent tuhercle hacilli and recovered.

With serums from tbe foregoing we tried to carry out the following plan of tests:

1. Effect of serum on healtby nnimals.
2. Treatment of tuberculous animals with serums to sbow influencc on course of disease nnd temperature.
3. Test of germicidal influence *in vivo* and *in vitro*.
4. Test of power to neutralize effect of tuherculin in small and fatal doses.
5. Test of effect on local reaction and temperature produced by tuberculin in tuherculous animals.

We must acknowledge at the outset that, for various reasons, we were unahle to carry out all of these tests with all of our serums, and the work is incomplete to that degree. Methods used in some tests were changed in others hecause tbey were thought to he fallacious, particularly in testing for anti-tuberculin. Consequently there is no true basis for comparison of all thc serums tried. In addition to the serums prepared hy ourselves, we have tried five or six from other sources. In the present uncertain state of serum-therapy for tuberculosis it is undesirahle to mention the names of their originators.

## Part I.

I. *Sheep injected intravenously with filtrate of cultures of tubercle bacilli on thymus bouillon.*

*June* 26, 1894. No. 1, treated, weight 64 lhs.; No. 2, control, weight 64 lbs. Both animals are "wethers" and in good condition. Began injections of filtrate of cultures of (non-virulent) tuhercle bacilli on calf tbymus bouillon into sheep No. 1. Four doses: June 26tb, 10 c.c.; June 30tb, 20 c.c.; July 3d, 25 c.c.; July 11th, 25 c.c. Temperature tbree bours nfter first dose 103.8.°

*July* 16*th*. Weigbts: No. 1, 52 lbs. (loss, 12 lhs.; no other effect noted); No. 2, 64 lbs.

*August* 4*th*. Weigbts: No. 1, 57 lbs.; No. 2, 67 lhs.

10*th*. Weights: No. 1, 56½ lbs.; No. 2, 67 lbs. No. 1 did not recover weight, and on this account hleeding was postponed.

*October* 27*th*. No furtber improvemeat or change noted. No. 1 hled 500 c.c. by canula. 175 c.c. serum collected. Tbis animal was so much weakened hy bleeding that it was killed ten days later. No lesions found nt autopsy.

*Effect of No. 1 Sheep-serum on the Course of the Disease and on the Temperature.**

*October 27th.* Took 6 guinea-pigs, average weight 523 grammes; (*a*) treated with serum, 4 guinea-pigs, average weight of 504 grammes; (*b*) controls. Before inoculation (*a*) received five doses of 4–5 c.c. of the serum subcutaneously; total, 24 c.c. each. No effect to be noted on temperature.

*November 5th.* Weights: (*a*) Treated, average 517; (*b*) controls, average, 511. All were inoculated in groin with virulent material from the lung of a rabbit in which tubercle bacilli were scanty. Each of (*a*) was injected with 4 c.c. of serum.

12*th*, 18*th* and 23*d*. (*a*) Injected with 5, 5, and 2 c.c.; total, 12 c.c. Serum pigs lost more weight than the controls. They showed no local irritation.

*Result.* Average time of death: (*a*) 44 days; (*b*) 62 days. Autopsies revealed generalized tuberculosis of all organs. The serum apparently hastened death and caused loss of weight in healthy animals before inoculation.

A preliminary test of the antitoxic power of this serum on pigs ninety-three days tuberculous showed that it did not prevent a temperature reaction after the use of tuberculin, and the outlook was so unpromising that further tests were abandoned. The bleeding of the sheep being postponed so long, the method may be said to have had hardly a fair trial.

II. *Fowls inoculated intraperitoneally with mammalian tubercle bacilli of increasing virulence.*

*September* 10, 1894. We took 12 fowls (chickens and cocks), two-thirds grown, having an average weight of 1026 gm. The first inoculation was of 1.5 c.c. of an emulsion of non-virulent tubercle bacilli from bouillon culture which had been grown four years in an incubator oven.

*October 27th.* Average weight 1671. Second inoculation: 0.7 c.c. of an emulsion of non-virulent tubercle bacilli from a bouillon culture which had been cultivated two years.

*December 20th.* Average weight 1717. Third inoculation: 1 c.c. of an emulsion of a virulent culture (cultivated four months on serum).

*January* 21, 1895. Average weight (10 fowls) 1686. Fourth inoculation: 2 c.c. of an emulsion of a virulent culture (growing five months on serum).

*February 15th.* Average weight (10 fowls) 2006. Fifth inoculation: 1.5 c.c. of the juice of crushed lymph nodes from a rabbit; third passage of virulent tuberculosis.

*April 27th.* Average weight (10 fowls) 1894. Sixth inoculation: 1.5 c.c. of the juice of the lungs and omentum; sixth passage of virulent tuberculosis.

*May 22d.* (Not weighed). Seventh inoculation: 1.5 c.c. of the juice of the spleen and omentum; eighth passage of virulent tuberculosis.

*July 19th.* (Not weighed.) Eighth inoculation: 1.5 c.c. of the juice of the omentum; seventh passage of virulent tuberculosis.

* Weight and temperature tables are omitted to economize space

*August 10th.* Average weight 1684. Ninth inoculation: 1.5 c.c. of the juice of the omentum; sixth passage of virulent tuberculosis.

*September 4th.* Tenth inoculation: 1.5 c.c. lung; eighth passage of virulent tuberculosis.

During the course of these inoculations only two of the fowls died, and those from injuries received in fighting. The rest were killed to death at various times after the seventh inoculation. Most of them were killed twelve and twenty-six days following the last inoculation. Only small amounts of serum were obtained, and part was preserved by trikresol. In no case was there found the slightest evidence of a past or present tuberculosis on post-mortem examination.

*Test of the Germicidal Power of Fowl-serum.*

*June* 15, 1895. We took an emulsion in water of the first culture of tubercle bacilli on serum (virulent). (*a*) Mixed one-half with 6 c.c. serum; no antiseptic added. (*b*) Mixed one-half with 6 c.c. of 0.6 per cent. NaCl solution. Both were allowed to stand six hours at room temperature in a dark closet. We then inoculated 3 guinea-pigs, average weight 358 gm., with (*a*) 2 c.c. each; and 3 guinea-pigs, average weight 345 gm., with (*b*) 2 c.c. each. All were inoculated subcutaneously in the right groin.

*Result:* All became tuberculous in the usual way: (*a*) lived 77 days; (*b*) lived 80 days. There was no germicidal influence *in vitro* on the tubercle bacilli.

The effect of this serum on the temperature of seven tuberculous guinea-pigs and rabbits was tried, with the result that doses of 1.5 to 2 c.c. seemed to cause some elevation in six hours. The amount of serum was insufficient for more of such experiments.

*Influence of Fowl-serum on the Course of the Disease in Guinea-pigs.*

*September* 20, 1895. We took 5 pigs, average weight 514.2 gm., for treatment; and 5 pigs, average weight 487.6 gm., for controls. All inoculated with virulent tuberculous material from lung of guinea-pig; each receiving 0.25 c.c. in the right groin. We began treatment the same day, using subcutaneous doses of from 1 to 2.5 c.c. every three to ten days, and giving a total quantity of 10 c.c. each. Abscesses formed in a few instances, probably from skin infection.

*Result*: The treated animals lived 57 days; the control animals 58½ days.

Tests of the temperature of these animals showed no perceptible influence of the serum. The limited quantity of serum available precluded tests with tuberculin. The results accord with those published recently by Auclair,[3] and were especially disappointing because fowls have such high natural immunity to mammalian tuberculosis and are able to dispose of large quantities of human tuberculous material.

III. *Sheep injected subcutaneously with increasing amounts of tuberculin.*

IV. *Sheep inoculated intravenously with living non-virulent tubercle bacilli cultures and injected with tuberculin in increasing doses.*

*April* 22, 1895. (III.) Sheep (wether), weight 70 lbs. (IV.) Sheep (wether), weight 65 lbs. Sheep (IV.) received 10 c.c. tubercle bacilli

from a non-virulent houillon culture in the saphenous vein. No harmful effects followed.

*May 16th*, 10 c.c. same. Weights: (III.) 105 lbs.; (IV.) 80 lbs. After this dose (IV.) lost weight and strength steadily during the summer, so that nothing more was attempted until December of the same year.

*December* 15, 1895. Weights: (III.) 105 lbs.; (IV.) 80 lbs. *Tuberculin Test.* Injected both with 0.200 tuberculin. The temperature of (III.) rose from 102° to 103.5°; (IV.) reacted from 102° to 105°.

*Tuberculin Injections.* During the next three months both sheep were injected with gradually increasing doses of tuberculin; at first every second day, then less frequently, according to the loss of weight. (III.) received 19 doses; the largest, 50 c.c.; the total quantity, 184 c.c. (IV.) received 23 doses; the largest, 20 c.c.; the total quantity, 64.5 c.c.

Both animals retained their weight until the maximum dose was reached, which for sheep (IV.) was evidently overwhelming, as it lost 15 lbs. during the following three weeks, finally becoming so weak and cachectic that it was killed. No tuberculous lesions were revealed by autopsy, but the liver and spleen were found much atrophied; there was a calculus in the pelvis of one kidney. The absence of tuberculous lesions and the apparent good health of this sheep up to the time of the tuberculin injections remind one of the effects mentioned later by Maffucci and Vesten[57] as the result of intravenous inoculations of living tubercle bacilli in sheep. The cachexia in one sheep was probably induced by the tuberculin injections. No signs of hæmoglobinuria were noted in these animals; this is mentioned by Niemann[58] as occurring after massive doses of tuberculin in goats, owing to the large amount of glycerin contained in it.

Sheep (III.) withstood the dose of 50 c.c. of tuberculin fairly well, and was killed March 21, 1896, five days later. 1000 c.c. were taken by canula from the external jugular vein. The weight decreased 20 lbs. during the following three weeks. Part of the serum was kept aseptic and the rest was preserved with camphor.

### *Test of the Germicidal Power of the Serum of Sheep (III.) Tuberculinized.*

*March* 23, 1896. We took mixtures of: (*a*) 6 c.c. of emulsion in 0.6 per cent. NaCl sol. of a washed sputum rich in tubercle bacilli, adding 12 c.c. of a serum without antiseptic. (*b*) 6 c.c. of emulsion of sputum as above; 12 c.c. of 0.6 per cent. NaCl solution. A stained drop showed one or two tubercle bacilli in a field. The mixture was placed in a cool, dark closet for six hours.

We then took 3 guinea-pigs, average weight 692, each of which received 6 c.c. of (*a*) subcutaneously, and 3 guinea-pigs, average weight 702, each of which received 6 c.c. of (*b*) subcutaneously.

*Result:* All became infected in the usual time, no difference in the course of the disease being observed. (They were therefore used for testing tuberculin later.) Autopsies revealed rather chronic tuberculosis in all. No germicidal influence was manifested by the serum.

### *Effect of the Serum of (III.) on the Course of the Disease in Guinea-pigs.*

*March* 23, 1896. We took 6 pigs, average weight 721 gm., and treated them with serum; 4 pigs, average weight 766, were taken as

controls. All were inoculnted in the right groin with one oese of washed sputum emulsion (same as in above experiment).* Injections of serum preserved with camphor were hegun on the following day. It was warmed and administered intraperitoneally to facilitate nhsorption. Doses of 2 to 5 c.c. were continued every second to fourth day for fifty days. The treated animals received total amounts varying from 50 to 65 c.c.

*Result:* By the twentieth day all the treated pigs were much more emaciated than the controls. One died from peritonitis from puncture of the stomach in injecting of serum. Three more treated pigs died in fifty days from tuberculosis, while the controls were still vigorous. The controls were therefore killed on the fifty-first day for comparison, and the lesions were found practically the same as in the treated. The serum appeared to act harmfully; at least when given in the peritoneum, though the two remaining treated animals survived 110 to 132 days, showing lesions which were chronic, but notsunusual in character. The effect on temperature was not noted in above experiment.

*Test with Tuberculin of Serum (III.) Antitoxic Power.*

Eight pigs inoculated with sputum were tested on the nineteenth, thirtieth, and thirty-third dnys of disease with serum and tuberculin mixed, given subcutaneously. The conditions of the experiments were so unsatisfactory that they deserve only brief mention. So far as could be judged, no favorable influence was observable. In some pigs the serum seemed to cause fever, and since the bleeding of the sheep was undertaken only five days nfter n large dose, it is conceivable that some of the tuberculin may have still been contained in the serum.

V. *Ass J.; inoculated intravenously with living, non-virulent tubercle bacilli.*

*December* 13, 1894. Male ass (J.); weight, 445 lbs. Appeared old, but sound. We injected 7 c.c. of an emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into an ear vein. (About one-third went into the subcutaneous tissue, producing induration and a cold abscess.) No effect could he noted on the health of the ass.

*January* 15, 1895. Weight 460 lbs. We gave successfully 15 c.c. of an emulsion of tubercle bacilli in 0.6 per cent. NnCl solution; the tubercle bacilli were taken from three bouillon tubes. No injurious effect was seen.

*March 7th.* Weight 480 lbs. We injected 15 c.c. of n similar emulsion of tubercle bacilli taken from 6 tubes, with partial success.

*April 22d.* Weight, 490 lbs. We attempted to inject 10 c.c. of n strong emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into the external jugular; most of it went into the subcutaneous tissues.

*May 16th.* Weight, 475 lbs. We gave 12 c.c. of n thick emulsion of tubercle bacilli in 0.6 per cent. sterile NaCl solution into an ear vein.

*Result:* Died in twelve hours from an embolus in the pulmonary artery. The emulsion was probably too thick and produced a clot. No evidence of tuberculosis of the lnngs, liver, or spleen could he found at

* Sputum was used for these inoculations, thinking more nearly to approach the infective power of tubercle bacilli for human beings.

autopsy, nor on microscopical examination of sections. Enough serum was saved from the heart cavities to test its bactericidal influence.

*Test of the Bactericidal Effect of Serum. Ass J. (V.).*

*May* 18, 1895. (a) 4 c.c. of the above serum, unfiltered, was mixed with 1 c.c. of the crushed and strained juice from the spleen of a tuberculous guinea-pig that had died in five weeks; (*b*) 2 c.c. 0.6 per cent. NaCl solution was mixed with 0.50 c.c. of the same juice. Both stood five and a half hours in a dark closet. 4 pigs, average weight 315 gm., received 1.25 c.c. each of (*a*). 2 pigs, average weight 293 gm., received 1.25 c.c. each of (*b*) in the right thigh.

*Result:* All became uniformly tuberculous as usual, indicating that the serum had no effect on the bacillus, though the experiment is obviously inconclusive.

VI. *An ass was inoculated subcutaneously and intravenously with virulent cultures of tubercle bacilli and virulent tuberculous material from animals; it was then injected with tuberculin in increasing doses.*

*February* 25, 1895. *Subcutaneous inoculations.* Female ass (R.); weight, 450 lbs.; full-grown, but young; in fine condition. The normal rectal temperature varies from 98.4° to 99.5°. The first inoculation was of 12 c.c. of an emulsion of the liver and omentum of a rabbit that had died in fifty-one days of acute tuberculosis (fourth passage through rabbits). There were few tubercle bacilli in the emulsion; a cheesy abscess resulted, which was opened and healed.

*April* 6*th.* Weight, 455 lbs. Second inoculation: 12 c.c. of an emulsion of the liver and omentum of a rabbit that had died forty days (fifth passage); tubercle bacilli were fairly numerous. An abscess followed.

*May* 22*d.* Weight 430 lbs. Third inoculation: 4 c.c. of an emulsion of the liver and omentum of a rabbit that had died in twenty-two days (seventh passage); tubercle bacilli were few. Injections were made in three or four places; no abscesses followed, but there was permanent induration.

*June* 9*th.* Fourth inoculation: 4 c.c. of an emulsion of the liver and omentum of a rabbit that had died in forty-nine days (seventh passage); tubercle bacilli fairly numerous. An abscess resulted; otherwise its health remained good. It was turned out to pasture for the summer.

*August* 9*th. Intravenous inoculations.* First inoculation: 5 c.c. of an emulsion of the lymph nodes and omentum of a rabbit three months tuberculous (seventh passage); it was injected into an ear vein; tubercle bacilli were scanty in the emulsion. No disturbance of health that was perceptible followed.

24*th.* Second inoculation: 2 c.c. of an emulsion from the lymph nodes and omentum of a rabbit three months tuberculous (eighth passage); tubercle bacilli were numerous. Following this the ass soon became daily thinner and weaker, and breathed somewhat quickly.

*September* 1*st.* Losing weight. Evening temperature 100.2°.

13*th.* Injected with 0.030 tuberculin; reaction to 104°.

*November* 19*th.* Gaining weight; evening temperature 100°.

20*th. Tuberculin injections.* Begun with 0.030, and gradually increased every two to ten days for five months. Thirty doses were given, aggregating 173.5 c.c. The last dose was 33 c.c. Her weight

gradually increased from 350 to 420 lbs., and the reactions became less. The last dose caused a temperature of 101°, but no depression. Little disturbance was noted from the tuberculin after the first four or five doses.

*May* 4, 1896. First bleeding: Ten days after the largest dose, 1500 c.c. of blood was taken from the jugular vein by canula. Some of the serum obtained was preserved aseptically in tubes, while the rest was kept with lumps of camphor added. No depression followed the bleeding, so the animal was turned out to pasture for the summer. During the summer of 1896 ass R. was quite thin, weighing only 345 lbs., but she had regained her weight in the autumn—to 400 lbs. on October 15th.

*November* 2*d.* Tuberculin injections were resumed, beginning with a dose of 0.500 c.c., which was followed by a rise of temperature to 102°. The doses were rapidly increased, causing about the same reaction, until December 29th, nearly two months. The last dose was 10 c.c.; the aggregate 46.5 c.c. An interval of thirty days was then given before bleeding, during which time the ass gained 20 lbs. in weight.

*January* 27, 1897. Second bleeding: 1800 c.c. was taken from the left jugular.

*Test of the Germicidal Power of the Serum of Ass R. (VI.).*

*First serum:* The same method was used as in the fowl and sheep serums except that virulent cultures were employed for infective material.

*May* 6, 1897. (*a*) 12 c.c. of serum (without antiseptic) was mixed with 6 c.c. of an emulsion of tubercle bacilli from a first culture on serum. (*b*) 12 c.c. of a 0.6 per cent. solution of NaCl was mixed with 6 c.c. of tubercle bacilli emulsion as above. Both were allowed to stand twelve and one-half hours in a dark closet.

We took 3 pigs, average weight 517 gm., and inoculated them with 3 c.c. each of (*a*); and 3 pigs, average weight 512 gm., which we inoculated with 3 c.c. each of (*b*).

*Result:* After twenty-six days the evidence of uniform disease was so palpable in all that they were killed by tuberculin. All were found to have lesions of generalized tuberculosis.

*Second serum:* This serum was not tried on animals, but was added to bouillon cultures without heat to test its inhibitive power on the growth of tubercle bacilli *in vitro*.

*January* 31, 1897. We took 4 flasks containing 50 c.c. each of bouillon + 10 c.c. or 5 c.c. of serum (R. second), 5 flasks containing 25 c.c. each of bouillon + 2.5, 1.25, 0.63, 0.50, and 0.25 c.c. of (R. second), 4 flasks containing 25 c.c. each of control bouillon + 10, 1.25, 0.50, and 0.25 c.c. of hydrocele fluid, and 1 flask containing 25 c.c. of control bouillon only.

All were planted with non-virulent tubercle bacilli, surface growth. Only one flask became contaminated. No retardation of growth was seen unless the serum was present in large proportion. In the latter case the alkalinity was so increased by the serum that from comparison with the hydrocele fluid cultures and from former observation we may attribute the lessened growth to the wide differences in this respect.

*Result:* There was no reason to suspect any antibacillary effect of a specific nature from the above experiment.

*Effect of the First Serum of Ass R. (VI.) on the Course of the Disease in Guinea-pigs.*

*May* 4, 1896. 10 pigs were inocnlated in the right groin with a virulent culture of tubercle hacilli, the first culture on serum. 6 pigs, average weight 500 gm., were treated with serum. 4 pigs, average weight 520 gm., as controls (*a*). 2 pigs, average weight 515 gm., as controls (*b*); which were not inoculated, but were treated. 1 pig, weight 541 gm., as control (*c*); which was neither inoculated nor treated, but was kept in the same cage for comparison.

Injections of serum into the peritoneal cavity were begun on the fourth day, and were continued every second or third day for fourteen doses, which ranged from 1 c.c. to 6 c.c. The average total amount for each pig was 45.7 c.c.

*Result:* The treated died on an average of 45.5 days after inoculation. Controls (*a*) died on an average of 42.5 days after inoculation; controls (*b*) died on an average 50 days after inoculation; control (*c*) gained 33 grammes weight.

No effect could be observed either on tho appearance of the treated animals or on their temperature and lesions as compared with the controls. The controls (*b*), which were to show the effect of the serum on healthy animals, were inoculated hy mistake May 20th. They serve, however, to show that the serum failed to prevent or modify the disease when given prior to inoculation. All died with generalized tuberculosis. The prolongation of life was not sufficient to he significant.

Possibly n more favorable result would have been ohtained with this serum had it heen used subcutaneously in smaller doses. The quantity at our disposal was too small to carry out a second test and to try its effect on tuberculous eyes of rahhits. The second serum from ass R. was thus tried, as will he seen hereafter. (The experiments on the antituberculin or antitoxic power of first serum, ass R., will he given in Part II.)

*Effect of the Second Serum of Ass R. (VI.) on the Course of the Disease.*

*February* 12, 1897. 15 pigs were inoculated in the right groin with a culture of tubercle hacilli of moderate virulence. 5 pigs, average weight 634 gm., were treated with the second serum. 5 pigs, average weight 602 gm., controls.* (*a*) 1 pig, weight 580 gm., control (*b*), was not inoculated, but was treated. 1 pig, weight 455 gm. control (*c*), neither inoculated nor treated.

Injections of serum were hegun next day under the skin of the ahdomen; doses of from 0.050 to 1 c.c. at intervals of one to three days were given until May 4th—nearly three months. No marked induration of skin nor abscesses resulted. The total for each pig was 17.4 c.c.

*Result:* 4 treated pigs died after an average of 95.2 days; 1 treated pig survived four months, and was then killed. 2 controls (*a*) died nfter an nverage of 75.5 days; 3 controls (*a*) survived four months and were then killed 1 control (*b*) lost 10 grammes in four months. 1 control (*c*) gained 15 grammes in four months.

No influence was manifest on the animals, nor on their temperatures taken before and after doses of 1 c.c., and compared with controls.

* The other five pigs were treated with Serum VII. See page 704.

Nothing unusual was noted in the lesions. All had chronic generalized tuberculosis.

This experiment was in direct contrast to that with the first serum in the less virulent inoculation and with smaller doses of serum given under the skin instead of into the peritoneal cavity. The serums seemed to have no favorable influence either way.

VII. *Ass injected subcutaneously: (1) with dead non-virulent cultures of tubercle bacilli on thymus bouillon; (2) with precipitated tuberculin made from cultures of non-virulent tubercle bacilli on proteid-free media; (3) with alkaline extract of tubercle bacilli mixed with dead tubercle bacilli; (4) with living non-virulent tubercle bacilli.*

*January* 4, 1895. Female ass (H.), weight 475 lbs., full-grown, rather "phlegmatic;" in good condition and apparently sound; normal rectal temperature—97° to 98°.

1. *Injections of non-virulent cultures of tubercle bacilli on thymus bouillon which had been killed by trikresol.* Began injections of emulsion of tubercle bacilli in thymus bouillon culture with 0.2 per cent. trikresol, on alternate sides of neck. January 4, 1895, 1 c.c.; 11th, 1.5 c.c.; February 8th, 5 c.c.; March 4th, 8 c.c.; April 8th, 10 c.c.

No rise of temperature followed these injections, and only indurated spots remained; no abscesses resulted. The animal was not visibly affected, except that she lost 15 pounds, which was regained by April 17th, when she was turned out to pasture for the summer. Nothing further was done until December 11th, when a dose of 0.500 tuberculin was injected without an ensuing reaction.

*December 12th.* 2. *Injections of precipitated tuberculin.* We began injections of toxin obtained from cultures of non-virulent tubercle bacilli on liquid media containing asparagin, mannit, or ammonium carbonate in mixtures with salts as in the formulas of de Schweinitz[84] and Proskauer and Beck.[70]

The toxin was prepared as follows: To the clay-filtered culture fluid 2 per cent. acetic acid was added, followed by ammonium sulphate to saturation. Nearly all the proteid matter arising from the fully grown cultures was thus precipitated. It was then collected, redissolved in water, reprecipitated by alcohol, filtered, washed with alcohol and ether, dried, and weighed. Solutions of the solid substance, 0.005 in 1 c.c., were made in weak sodium carbonate. 0.5 c.c. of this preparation was not fatal to tuberculous guinea-pigs, though producing high temperature and local irritation at the site of injection.

This pure tuberculin or toxin was injected every second day in increasing doses up to 7 c.c. until January 30, 1896. The total quantity was 26.35 c.c. Abscesses then formed at site of injection, and occasionally thereafter from the following treatment:

3. *Injection of alkaline extract and dead tubercle bacilli.* The toxin was next prepared by adding 1 per cent. NaOH to the cultures before filtration; warming to 40° C.; filtering through cotton; precipitating by 2 per cent. acetic acid, which gave an abundant flocculent substance; filtering again; washing out the acid with water by decantation; re-solution in weak alkali. Naturally this fluid contained many dead and disintegrated tubercle bacilli, as the filtration was carried through cotton and paper. We found it impracticable to filter through clay, since most of the dissolved poison was left on the filter.

From February until June, 1896, fifteen injections were given subcutaneously, in increasing doses, of these solutions well diluted and freshly made. The largest amount represented 3500 c.c. of cultures. In the aggregate, 17,500 c.c. of cultures grown in 100 to 150 c.c. flasks were used. Considerable induration persisted after these injections, and on three occasions they were followed by septic abscesses which necessitated an interruption of the treatment. The solutions not being sterilized, and the dissolved poison and dead bacilli both being such strong irritants, we despaired of further increasing the doses. Throughout the time of treatment the ass had but little disturbance of health except at the time of the abscesses, when she did not eat well. The abscesses were opened as soon as formed, and healed promptly. By June 1st she had lost fifteen pounds, but regained it by the 15th inst.

*June* 24, 1896. First bleeding: Nine days after the last dose of toxin and tubercle bacilli, 1000 c.c. of blood was drawn from the right external jugular vein; 400 c.c. of serum was obtained aseptically. All the abscesses had healed at this time except two or three small pustules. The animal was then put into the pasture. Although this had been an unpromising experiment, owing to the abscesses, we decided to try the serum, omitting the germicidal test on animals.

*Effect of First Serum, Ass H. (VII.), on Course of Disease in Guinea pigs.*

*June* 26, 1896. 10 pigs inoculated in right groin with tuberculous material from a guinea-pig; virulent infection. 6 pigs, average weight 710.8, treated with serum; 4 pigs, average weight 713.7, (*a*) controls; 2 pigs, average weight 780, (*b*) controls, not inoculated, but treated with serum.

The serum (with camphor added to insure preservation) injections into peritoneum were begun on next day and given every second day for a month with doses of 1 to 7 c.c.; total quantity, 41.5 c.c. each.

*Result:* Average time of death: treated 47 days; (*a*) controls 49.7 days. Controls (*b*) lost weight and were inoculated later, but developed the disease in usual way. The temperatures of all were taken on the twenty-second day of disease and after the dose of 7 c.c. serum, without showing any influence from serum. The antituberculin tests will be found with the other serums in Part II.

4. *Ass inoculated subcutaneously with non-virulent living cultures.*

*November* 7, 1896. Took ass H. (same as was used in VII.) after being in pasture all summer. Weight 490 lbs. Indurations from former treatment remained, but were smaller.

The first inoculation was of 25 c.c. of culture fluid with tubercle bacilli rubbed up in mortar, weighing in moist condition 0.0221—from an actively-growing culture of non-virulent tubercle bacilli on acid bouillon. The injection was into the right shoulder. The temperature six hours later was 99°.

*November 20th.* Second inoculation: 25 c.c. of a culture containing 0.1353 tubercle bacilli into left shoulder. Several small aseptic abscesses from the first inoculation were to be seen.

*December 7th.* Third inoculation: 25 c.c. (the tubercle bacilli were not weighed, but more were used); the injection was made into the right groin; there was no abscess from last dose.

*19th.* Fourth inoculation: 40 c.c. into the left groin; no abscess resulted.