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ANTITOXIC AND BACTERICIDAL PROPERTIES OF THE SERUM OF HORSES TREATED WITH KOCH'S NEW TUBERCULIN T. R.

BY DR. C. FISCH, ST. LOUIS, MO.

All attempts at establishing a sero-therapy in tuberculosis have hitherto failed, that is to say, if by sero-therapy we understand a specific prophylactic or curative treatment, like the one established in diphtheria or tetanus. So decided and pronounced has this failure been, that in the mind of many observers a strong prejudice has been created, a prejudice that appears the more justifiable since commercialism has not hesitated in exploiting so promising a field. We would, however, be very hasty in drawing from these failures the conclusion that sero-therapy in consumption is not a thing to be hoped for, as has been done by many writers. We cannot be too chary in making final statements, a postulate that at this very time emphasizes itself with particular weight by the discovery made by *Kitasato* of an antitoxic typhoid serum. Has it not been for sometime past one of our axioms, that in typhoid and cholera only bactericidal and not antitoxic properties were developed? The less we try to encase new ideas—and sero-therapy is a new idea—in the form of old conceptions, the less frequently shall we have to retrace our steps. No mete-wand has as yet been discovered to point out the limits and boundaries of this new idea;

whoever talks about the limits of sero-therapy, confesses that he does not, or will not, understand its origin.

For these very reasons it is an unpromising task to foretell by logic reasoning, whether or to what degree serum treatment will influence tuberculosis. Nobody ever expected that it would bring about a *restitutio ad integrum* in advanced cases, or redress the ravages of septic complications. It is certainly absolutely erroneous to insist that the anatamopathologic phenomena of pure tuberculosis are not indicative of a toxic process which might be amenable to antitoxic impressions. Such intoxication exists from the very first hour, I might say, of the beginning of tuberculous infection, a fact that is not only evidenced by histiologic changes, but that can also be proven by physiologic reactions. To say that the early tuberculous changes are simply of an inflammatory character expresses only a morphologic phenomenon. Physiologically they are as pronouncedly caused by bacterial toxins as are any other bacterial intoxications. It is true, this toxic inflammatory process is little noticeable in its early stages and becomes mostly chronic, gradually undermining the vital vigor, and leading to fatal loss of substance, or secondary infections (in fact, very few persons die of chronic tuberculosis), but in this very chronicity the battle waged between the human organism and the array of bacterial toxins expresses itself; very often, perhaps in the majority of cases, the former remains victorious. In our language such a victory means immunization, although only for the time being.

This is not the place to enter more fully into these exceedingly interesting questions. I had to touch upon them because it has ever and again been contested that, theoretically, there were no prospects for sero-therapy in this disease. Valu-

able remarks regarding this topic may be found in an able paper by *Joseph McFarland* (1).

But we should be wrong if we attributed this reservedness to well conceived and understood pathologic conditions alone; behind them there lurks a preconceived idea of self-limitedness in, and above all a mysterious predisposition for, the disease. This hereditary predisposition would be well worth looking into a little more thoroughly; I cannot do it here, and must confine myself to the statement that a scientific basis for the tubercular predisposition is still wanting (2).

Without mentioning those attempts at putting forth an antitubercular serum that were prompted only by mercenary motives, the number of honest workers in this field is quite large. Since *Richet* and *Hericourt* first began their experiments, an enormous amount of persevering and unselfish work has been done. I will only mention the names of *Courmont*, *Babes*, *Niemann*, *Maragliano*, *Schweinitz*, *etc.* Admitting that there are considerable differences between the several methods employed by these investigators, to enter into which would lead us too far here, there is one source of error common to all, and which is to be found in the material used for immunizing the animals which were used for the purpose. If we look over the laborious experiments of *Koch* to establish immunity by means of tubercle bacilli or of their products, we find that the chief difficulty was to cause the tubercle bacilli to be absorbed by the tissues, or to chemically so act on the bacilli that their immunizing ingredients were contained in a fluid extract. The peculiar morphologic and

(1). Journal of Amer. Med. Ass'n, 1897, Aug. 21, p. 239.

(2). *W. Freudenthal*, in an article on the aetiology of pulmonary tuberculosis. Annals of Otology, Feb. 1897, makes some very appropriate remarks on this point.

chemic constitution of the tubercle bacilli made indeed all these experiments unsuccessful. *Koch* had finally to destroy the morphologic entity of the bacilli in order to obtain unobjectionable results. In other words, the essential toxins (some writers, it is true, assert that there are no essential tubercle toxins) are not excreted, as they are in cultures of diphtheria bacilli, but are to by far the greatest extent enclosed within the membrane of the bacterial cell. The culture fluids contain none, or at least very little, of these specific toxic substances; they may be injected in comparatively large doses into animals without causing serious damage (3). We may add right here that it seems that there are produced a number of different toxic substances—this, at least would best explain the contradictory results of some observers; but we must not forget that ferments or enzyms like the bacterial toxins are very unstable compounds, and are easily changed in their chemic nature by all kinds of artificial procedures. It is, therefore, more than likely that the different bodies isolated from tubercle bacilli, or from their culture media, are not pre-existing, but represent ingredients of the former in a changed form.

This certainly holds good for the old tuberculin which has mostly been used as an immunizing agent. How little this fluid represents the active principles of the live tubercle bacillus is well illustrated by the following observation: If some of the dead and extracted bacilli which remain as residue after the preparation of the tuberculin are injected into suitable animals, they not only cause the formation of typical tubercles, but even prompt these animals to yield a typical tuberculin reaction. It is well known, furthermore, that this

(3). *Strauss* and *Gamaleia*, Arch. de Med. Expér., 1891. *Mafucci*, Centralbl. f. allgem. Pathol, 1890.

tuberculin reaction, the nature of which has by no means as yet been explained, may be obtained by several other similar bacterial compounds (proteins), and even by heterogeneous albuminous bodies (deutero-albumoses). No matter in which way the tuberculin be prepared, it is *a priori* impossible that the serum of animals immunized against it should possess any antitoxic properties, so far as the tuberculosis toxins are concerned. A certain anti-tuberculinic power may be exhibited by it (this is apparent especially by experiments made by *Schweinitz* (4), *Babes* (5), and others), and by it some influence on tuberculous processes may be, now and then, explained. In general, the results obtained with such a serum have been discouraging. The same obtained for those modifications of anti-tubercular sera, for the preparation of which, in addition to the tuberculin, cultures of the bacilli, dead or alive, have been utilized. As said above, these bacilli are very slowly resorbed; the greater part of them is ejected in the pus of the abscesses formed at the site of the injection. It must not be denied, however, that in some instances the results thus reached were fairly promising. This refers especially to the work of *Niemann* (6), and *Babes* (7), who really repeatedly immunized guinea pigs in this way against inoculation with living bacilli. This proves that the serum prepared by them possessed distinct antitoxic (according to *Babes*, even bactericidal) properties. But they also operated only with a part of the active principles of the tubercle bacillus,

(4). New York Med. Journ., July 24, 1897, and Journ. Amer. Med. Ass'n, July 17, 1897.

(5). Zeitschrift fur Hygiene u. Infectionskrankheiten, XXIII Heft III, p. 367.

(6). Bacteriolog. Centralbl. 1896, and Münchener Medicin. Wochenschrift, 1897, No. 3.

(7). l. c., see also *Redon* and *Chenot* in Comp. rend. Soc. d Biol., January 12, and June 29, 1895.

and accordingly the outcome of their labors lacks consistency and certainty. In no case out of the great number of respective investigations was a stage reached in which the observer could with certainty foretell the result of an experiment.

That both antitoxic and bactericidal potencies must be qualities of an anti-tubercular serum is shown by the well known phenomenon, that we have to deal sometimes with an infectious, mostly with a toxic type of the disease. That living bacilli, furthermore, as such, are less apt for the production of such a serum than dead ones (that is to say than bacilli more easily accessible to disintegrating influences) is indicated by the enormously larger toxicity of dead bacilli ([8]). It may be well in this connection to mention the important fact that from the tuberculous organs of infected animals an extract can be prepared which, while being exceedingly toxic, in a short time immunizes guinea pigs if injected into them in gradually increasing doses, against the introduction of virulent cultures ([9]).

If so, it goes without saying that "anti-tuberculin sera" are not what must be claimed for an anti-tubercular serum, there are certain direct drawbacks attached to them that under circumstances have proved very obnoxious. In the first place, the assimilation of the tuberculin by the animal organism is a slow process; it may happen that the serum of these animals contains unchanged tuberculin instead of anti-tuberculin. The following experiment illustrates this fact: A healthy guinea pig received in seven doses, during twenty days, as much as 10 cc. of tuberculin.

(8). *S. Pansini*, Alcune osservazioni sulla tubercolosi, especialmente sulla tossicita del suo bacillo. Giorn. internaz. de Scienze Mediche, Anno XVII.

(9). See *A. Maksutow* in Bakteriol. Centralbl., XXI, p. 317.

Ten days after the last injection some blood was drawn from this animal; 1 cc. of the serum of this blood was sufficient to produce a typical and very violent tuberculin reaction in a tuberculous guinea pig. This phenomenon is at the bottom of what in the literature of the subject is known as transmitted tuberculin action. Its undesirability is evident.

Again, it is little known that bacterial proteins in glycerinic extracts, when administered for any length of time, invariably tend to produce a chronic nephritis. *Niemann* (10) first called attention to this fact, and I found it confirmed in two horses which for some months had been treated with high doses of tuberculin. It is not impossible that the fatal effects which these sera sometimes have on animals, are due to uræmic products retained in the blood serum. *Babes* (11) saw guinea pigs die from the injection of ½ cc. of such a serum; *Rutkowski* (12) relates similar accidents in his report on *Vicquerat's* anti-tubercular serum. I, myself, repeatedly saw guinea pigs die in a very short time from the injection of ½ to 1 cc. of *Paquin's* serum. The autopsy (death in diastole) did not reveal any definite cause of death.

The foregoing somewhat lengthy remarks were necessary in order to show that the solution of the tuberculosis serum problem depended upon the discovery of some means of making the tubercle bacilli with all their constituents easily resorbable. I did not fail at once to see that with *Koch's* new Tuberculin T. R. (13) this means was given, and I immediately set to work to follow out this idea. But I would not like to be understood as claiming this work to be something original; logically the

(10). Bakteriol. Centralbl., XVIII, p. 126.
(11). l. c., p. 362, etc.
(12). Bakteriol. Centralbl., XXI, p. 74.
(13). Deutsche Medicin. Wochenschrift, April 1, 1897

thought was bound to offer itself, and, in addition to this, *Koch* himself had insinuated it. While my experiments were going on, I had the satisfaction to hear that *Behring* (14) was following the same lines, though no details whatsoever about his investigations have as yet reached this country.

It need not concern us here, that at the present time a hot war is raging as to the therapeutic value of this new tuberculin; in contradistinction to other observers (15) I can fully confirm *Koch's* statement about its immunizing properties towards animals. But the salient point was that, at last, in it we had a substance which exhibited in full, and unchanged, all of the toxic ingredients of a tubercle bacillus. We have seen before, that the culture media of tuberculosis cultures contain only a very small amount of toxic bodies and those most likely the outcome of disintegration of the bacilli (16).

If, therefore, a conclusion *per analogiam* were allowed, this new tuberculin (T. R. I shall henceforth call it) by immunizing animals would be likely to produce the desired antitoxic effects, if such effects could be obtained altogether. Within the limits of our present knowledge, no other means of doing this could be conceived (17). I began experiments in this direction, and the following remarks will give their results.

According to *Koch's* directions, the greater part of the tuberculous toxins is excluded under the name "T. O."; this T. O. contains those toxins that are contained in the adhering traces of culture media, as well as those that are easily extracted

(14). See Berliner Klin. Wochenschr., 1897, July 12, No. 28; and Deutsche Medicin. Wochenschr., 1897, June 17.

(15). For instance, *Trudeau & Baldwin* in Medical News, Aug. 28, 1897, etc., etc.

(16). cf., however, *Pansini* in Bacteriol. Centralbl., XXII, p. 188.

(17) *Buchner* seems to have achieved the same or a very similar result by a different method.

from the membranes of the bacilli. They do not seem to have a great immunizing power, but are exceedingly toxic, and in many respects resemble in their effects the old tuberculin. But try as I might, I could not convince myself as to the logic necessity of excluding this part of the toxins from the immunizing process, and much less so since in my animals I had no occasion to fear any, not to say, very severe, reactions. The same, I thought, applied to the fraction of toxic substances contained in the culture media. To express myself candidly, I did not want to take any chances of omitting some toxic body that afterwards might prove of intrinsic value, although I knew that T. R. was of itself sufficient to immuninze my animals against these substances. I used only strong and perfectly healthy horses ([18]).

The way in which I proceeded may consequently be described as follows: I began with the injection of 1 cc. of T. R., which did not cause any reaction whatsoever; in the subsequent injections which were made about every seven to ten days, the amount was about doubled each time until 30 cc. of pure T. R. were reached. The reactions so far were very slight, the temperature never rising more than 1½° F. At this stage I commenced adding small amounts of T. O., as well as an aqueous extract of the nutrient agar; the reactions were very severe, sometimes reaching perfect prostration. Mostly a profuse diarrhœa set in; after a temporary fall, the temperature rose to 104° and 105°. Loss of appetite occurred, etc., etc. In four or five days these symptoms disappeared. Sometimes abscesses formed, caused by some undestroyed tubercle bacilli. Gradually and cautiously I reached a combined dose of 75 cc. of T. R. and 30 cc. of T. O. The indications are,

([18]). Tuberculin and Mallein test negative.

however, that much higher amounts will be tolerated, and I do not propose to stop before external reasons call for a halt. Both preparations, T. R. and T. O. were prepared in my laboratory, and the culture I used was of such a virulence that the *dosis minima* when injected into the abdominal cavity of a guinea pig (about 500 grms.) killed the animal within ten to fifteen days. As *dosis minima* I came to consider one loopful of an emulsion obtained by thoroughly triturating one loopful (1 mg.) of a four-weeks' agar culture with 1 cc. of sterilized water. Smaller doses produced a protracted course of the disease which then invariably attacked the lungs. The toxic power of this culture was so great that about 16 mg. of the dried and finely triturated bacilli killed within twenty-four hours guinea pigs of the above average weight. Therefore, if with Behring ([19]), we call m. the fatal dose of toxin for one grm. of guinea pig, my culture possessed a toxicity of 30,000 T. m. I tested its toxicity during several generations, but did not find any material deviations. Cultures of a different origin showed only a potency of 400 and 2000, respectively. A culture of aviary tuberculosis goes as low as 260. Of course we must not lose sight of the fact that these figures only hold good for guinea pigs.

The first blood was drawn after a T. R. dose of 50 cc. (+25 cc. of T. O.) was reached, and with the serum obtained from this blood the following experiments were made. I will remark, however, right here, that the serum from later bleedings (made after larger doses of the immunizing fluid had been administered) showed in all essentials the same characteristics, only in an intensified degree. For my experiments I used guinea pigs, monkeys and, to a certain extent, rabbits. I pre-

([19]). l. c.

served my serum by the addition of camphor, of .5 per cent phenol or .3 per cent trikresol. The action of the serum on a healthy animal is only noticeable by a slight fall of temperature two or three hours after the injection (Table I); this fall of temperature ranges from ½° to 1°. Comparative experiments (Table II) with normal horse serum, demonstrated, however, that here, too, the same phenomenon could be observed. The amount injected varied from .25 cc. to 2 cc. Otherwise no local or general reaction occurred.

In order to determine the possession of any immunizing power by our serum, in one series of experiments (Table III) five guinea pigs were treated during thirty days with repeated injections of .25 cc. of the serum; they received altogether 4 cc. of the serum each. After thirty days three of them were inoculated with the fatal dose of tubercle bacilli, the two remaining ones being kept as controls. At the same time two fresh animals received the same fatal dose of bacilli. The result was that after twenty-four and twenty-one days, respectively, the two non-treated animals died with the typical lesions of tuberculosis, while the three inoculated ones, as well as the two controls, remained healthy and continued to gain in weight, the same as they had done during the serum treatment. After six weeks one of the infected animals was killed; the autopsy did not reveal any lesions whatsoever, neither at the point of injection nor elsewhere.

Two other sets of five animals each were subjected to similar conditions, only the amount of serum and the number of injections varying. One series (Table IV) received during thirty days five injections of .25 cc. of serum each. Of three animals of this series inoculated with the fatal dose of tubercle bacilli, one died after twenty

days; the two others showed extensive infiltration around the point of injection, but no ulcerations. They did not lose in weight. Whilst this experiment shows that the quantity of serum used was insufficient for complete immunization, its effect is, nevertheless, very apparent.

The third series was treated during the same length of time with five injections of .50 cc. each. Then three of them were inoculated. All animals remained well and continued to gain in weight (Table V).

Though these experiments comprised only seventeen animals, the evidence brought out by them may well be considered conclusive, in view of the fact that even an intraperitoneal inoculation (Table III, No. 21), which otherwise invariably results fatally in a short time, did not cause any lesions whatsoever.

Naturally, as the next step in my investigations, the question offered itself, how the serum would influence tuberculous infection when applied at the very moment of infection. Various amounts of serum were therefore mixed with a fatal dose of bacillary emulsion, the mixture being injected subcutaneously, or into the abdominal cavity. Of five guinea pigs which in this way received 1 cc. each, not a single one showed any signs of infection. The same results obtained for six others into which ½ cc. each was injected, and for the third set of three to which was given .25 cc. each. When I lowered the dose to .10 cc. the results became valueless (Table VI). As controls for this experiment served two sets of three animals each, one being simply inoculated and left without treatment, while the second set received with the bacilli .25 cc. of *Paquin's* Antitubercle Serum. All six animals died within the usual time, two of the last *(Paquin)* set before the others.

We shall see later on that our serum possesses very decided bactericidal properties. For this reason the experiments of the last series had to be varied so that bacilli and serum were injected at the same time, but each for itself and in a different place. In this way three guinea pigs were treated, receiving the virus on the back, and the serum (.50 cc.) on the abdominal aspect, as nearly as possible at the same time. They remained in perfect heath, except No. 12, which showed, eight days after inoculation, a slight enlargement of the inguinal glands. The latter disappeared, however, in a short time, and the animal is as healthy to-day as are its mates (Table VII).

After these tests the most difficult problem remained to be solved: How far advanced may a case of guinea pig tuberculosis be and yet be amenable to a curative treatment by this serum? The number of experiments bearing on this question is, I confess, but limited. But I convinced myself that with almost absolute certainty one succeeds in saving the life of the animal, when treatment is begun within the first ten days after inoculation with my culture. This time-limit reached, the results became uncontrolable. I instituted treatment in several series, four, seven and ten days, respectively, after inoculation; injections (uniformly .25 cc.) were given regularly every other day for four weeks; after that time one injection per week was deemed sufficient. Of eighteen animals I have lost, until the present date, not a single one by the disease, though in one an enormous glandular enlargement has developed. The characteristic ulcerations at the sites of inoculation are absent, and the temperature is normal.

Although I would not like to appear over-confident (the time elapsed since treatment was begun

being only two and one-half months), I may safely assert that I consider these animals as recovered. In three of them I made the routine tuberculin test after six weeks of treatment without obtaining a reaction (Tables VIII to XI). Wherever ulcers had formed before the treatment they healed readily, no symptoms remaining to indicate a pathologic condition. The temperature was easily reduced to normal (Table XII); the weight kept steady or increased slightly.

Two animals of series X were sacrificed afer six weeks to enable me to study the pathologic anatomy of the diseased organs. The liver showed those peculiar cicatricial ridges described by *Koch* as characteristic after T. R. treatment. The spleen in one case was extraordinarily contracted, etc. In one word, everywhere successful attempts at restitution or at least encapsulation were obvious, the latter especially in diseased lymph glands. A description *in extenso* of these very interesting changes I must reserve for some future time, after my animals have been observed for a longer period. As controls, I used again some animals simply inoculated with bacilli, and others which in addition received the benefit of the *Paquin* treatment. All of these animals died in due time.

For brevity's sake, I omit to mention a number of other experiments destined to investigate the protective and curative potency of the T. R. serum. Those that have been reported are more than sufficient to establish the fact that this serum not only protects guinea pigs against infection, but that it is, too, of a very powerful curative potency.

Very gratifying also was the outcome of some experiments made on monkeys (belonging to the genus *Cebus*, of the order of the *Platyrrhini*). On July 22nd, two of them were inoculated with .25 cc. of bacillary emulsion into the abdominal

acvity. While one served as control, the other was treated to regular injections every other day of .50 cc. of T. R. Serum; this treatment was begun the day after inoculation. Very soon in the control animal high temperature set in (rising to 105° F. and 106° F.), emaciation became visible, and on September 10th, death occurred from the most extensive visceral tuberculosis I ever saw. The lungs were not affected at all. His more fortunate mate is to-day alive and healthy, and did not at any time exhibit any symptoms of infection. His temperature remained perfectly normal, his weight increased slightly, and some days ago he did not react at all after .15 cc. of the old tuberculin was injected subcutaneously.

Very satisfactorily, too, resulted an experiment on two other monkeys, into the trachea of which, after tracheotomy had been performed, .25 cc. of bacillary emulsion was injected. Treatment of one of them was begun immediately after inoculation (Aug. 16th.) in the way described in the former experiments. The control animal died September 5th with very extensive lesions of the larynx, lungs, liver, and the whole lymphatic system; an enormous tuberculous ulcer had developed at the very place where tracheotomy was performed. Emaciation was very marked, as well as the anæmia. In contradistinction, the other animal kept a steady temperature and weight, and offered no sign of disease, except a small ulceration of tuberculous nature at the place of incision; I feel sure that prolonged treatment will cure him entirely.

For certain reasons I would like to omit here a report of experiments with intra-ocular inoculation of rabbits. The well-known uncertainty of such experiments arising from very marked differences in the susceptibility of these animals, is an

element that prevents conclusive deductions. It may, however, be said that when inoculation and serum injection were practised at the same time in no case was an infection effected. The results varied whenever four or more days intervened between the two.

However tempting and alluring the reported results may appear to one uninitiated into the deceptive phenomena and phases of experimental tuberculosis, it is necessary to accentuate the fact that *per se* they do not form conclusive evidence so long as the animals have not been observed for a long period (five to eight months). In order not to be misunderstood, I must repeat that the period of my observations extends over only two and one-half months. But combined with the following considerations they form an absolutely safe stronghold: If the T. R. Serum acted specifically this must be due to its possession of antitoxic or bactericidal properties; it became necessary, therefore, to demonstrate the latter.

The slowness of growth of tubercle bacilli, as well as their peculiar cultural arrangement, compelled me to submit them to the following procedures, which entirely differ from the usual method of determining the bactericidal power of a fluid. A number of sterile test tubes were filled each with 5 cc. of fresh T. R. Serum. A few drops of an emulsion of tubercle bacilli were added to each of these tubes, whereupon the whole series was put into the incubator. After the lapse of a certain time the single tubes were removed and 1 cc. of their contents injected intraperitoneally into healthy guinea pigs. Table XIII gives the results *in nuce*. It was found that a contact of the tubercle bacilli with the serum during five hours was sufficient to destroy their vitality, or at least to impair their power of resistance so

much that the animal organism could easily rid itself of them.

The only attempt at determining this bactericidal power of an antitubercle serum that I could find in the literature was made by *Babes* ([20]), but the bacilli in his case were killed only after a contact of twelve days' duration. Of course I do not know but what the addition of a preservative will decrease this power in my case to a certain degree.

In the search for antitoxic properties, the customary method of combining the serum with a certain amount of old tuberculin (either fatal or just sufficient to bring about the characteristic tuberculin reaction) offered itself first. *Babes* and *Schweinitz* in this way proved the "antitoxic" nature of their sera. So did *Niemann*. But, according to what has been said above, the only thing proven by these tests is their antituberculinic nature, which of course will be present, too, in a really antitoxic serum, but at the best only forms a part of its potency. That, by the way, not even this antituberculinic quality is possessed by some of the antitubercle sera has been shown by *Behring* (l. c.) who found that *Maragliano's* serum is perfectly void of it. As to *Paquin's* antitubercle serum, I repeatedly came to the very same negative result.

How my T. R. serum behaves in this regard will be seen from Table XIV. I found that .50 cc. of it is sufficient to save a tuberculous guinea pig from the fatal dose (.20 cc.) of tuberculin, and that .1 cc. prevents the tuberculin reaction (.10 tuberculin). The tuberculin that I used was of a strength that 1.5 cc. killed a 500 grm. healthy guinea pig within twenty-four to forty-eight hours; I prepared it from cultures of my virulent bacilli.

([20]). l. c., p. 365.

Niemann for his serum found the relation 7 : 1, while in our case it would be 1 : 1.

But since it is evident that tuberculin did not mean tuberculous toxin, I worked out another method, which with due regard to the incompleteness of our knowledge gave very satisfactory results.

I profited by *Koch's* investigations, combining T. O. with T. R. and thus getting a fluid which contained in an absolutely unchanged form all of the substances (toxins, etc.,) the effect of which on the animal organism was to be studied. After the whole of the bacilli had been thoroughly triturated, I gauged the suspensions of them so that every cc. of the 20 per cent glycerine solution contained 1 mg. of solid substance. Doses up to 8 and 10 cc. of this fluid were borne by healthy guinea pigs without any trouble, while higher doses produced irregular fever and infiltration, and 15 to 16 cc. invariably caused death.

Quite different was its action on tuberculous animals; one mg. always produced within thirty-six hours an extended inflammatory infiltration, and a strong fever reaction differing from the tuberculin reaction, inasmuch as the rise of temperature appears rather sudden (two or three hours after injection) and keeps steady for ten to twelve hours, after which time a decline by lysis occurs. The infiltrations disappear usually within three days. Very characteristic and interesting is the fact that in one and the same animal such a reaction may be elicited indefinitely, by only a slight increase of the dosage (Table XV). The common tuberculin reaction, in the same animal, usually fails the third or fourth time. I do not yet know what influence our reaction has upon the tuberculous lesions; existing ulcers heal readily.

If instead of 1 mg. we use 2 mg. this dose invariably produces death within twenty-four hours.

Before we can utilize this toxin for determination of the antitoxic value of our serum, one objectionable feature of the test will have to be removed, viz: the inequality of the extent of the tuberculous lesions in the animals used. Although in guinea pigs the disease partakes of the character of a self-limited trouble, the extent of the lesions and the constitutional conditions vary enormously in different animals inoculated at the same time. The reactive capacity, naturally, varies with these conditions so that, although for qualitative tests any animal, provided it be tuberculous, will be satisfactory, this is not so for quantitative determinations. Here we must be as far as possible sure to always encounter the same power of, or rather lack of power of, resistance in our animals.

The beautiful investigations of *Borrel* (21) and *Kaspareck* (22) furnished the material to obviate this difficulty; while the former demonstrated the fact that as soon as thirty-six to forty-eight hours after inoculation tissue changes become observable, the latter added the valuable information that for the appearance of the tuberculin reaction such tissue changes are necessary, and that this reaction may be typically observed about thirty-eight hours after infection. The eminent theoretical importance of these two facts is apparent (perhaps especially with regard to an alleged pre-tuberculous stage of the disease); I found in them a means to procure for my tests a nearly always equivalent material. If into healthy adult guinea pigs of about the same weight (500 to 700 grammes) always the same amount of virus (one loopful of my culture) be injected, and if at a stated interval afterwards (forty-eight hours), the same amount

(21). Tuberculose pulmonaire expérimentale, Paris, 1896.

(22). Wiener Klinische Wochenschrift, 1897, No. 26.

of T. O. and T. R. toxin, together with the serum to be tested, be administered, we have done as much as can be done in order to obtain comparable results.

Preliminary experiments showed me that 1 cc. of my toxin injected into these forty-eight-hour guinea pigs elicited the above described reaction, together with a very marked infiltration, while 2 cc. were found to be the fatal dose here, too.

By such experiments I knew also that less than 1 cc. of my serum inhibited all these reactions. If, therefore, we agree to call an antitoxic unit 1 cc. of that serum which counteracts 1 mg. (1 cc.) of toxin (always supposing that the serum has been prepared by means of the same race of bacilli from which the toxin is derived), it is easy to determine the potency of the serum under discussion. Accordingly a number of guinea pigs of about 500 grammes weight were prepared in the way described, whereupon various amounts of serum, each mixed with 1 cc. of the toxin, were injected after forty-eight hours. While .3 cc. were not able to materially influence the reaction, with .4 cc. no temperature reaction, nor local infiltration occurred; .6, .8 and 1 cc. acted in the same way. This means that the serum is 2.5 times more active than a normal antitoxic serum; in other words, that it represented 2.5 antitoxic units to the cc. This seems to be of very low value when compared with the potency of other antitoxic sera. But the serum of the same horse one month later, after immunization had been continued all the time, showed a potency of 3.7. The serum of another horse was found of a strength of 2.8 the first time, of 4.1 six weeks later. These findings I believe to be the most valuable part of my work since they justify the hope that in due time a serum of very high power may be obtained.

On the other side, we must not forget that our serum is not only antitoxic, but in a very high degree also bactericidal, the latter quality being, under certain circumstances, probably more valuable than the former. Furthermore, I think it highly probable that later on a tubercle virus may be obtained of much greater toxicity. Some experiments are under way to find out whether, after a method similar to that of *Metchnikoff*, *Roux* and *Salimbeni* ([23]) (viz: cultivation of tubercle bacilli enclosed in small pouches of collodion, which are introduced intraperitoneally into guinea pigs or rabbits), still more virulent forms may be obtained. Be that as it may, my researches so far have demonstrated the fact that a serum both antitoxic and bactericidal may be *obtained by immunizing horses against tuberculosis by the new tuberculin T. R., and that it is possible to immunize (and cure) guinea pigs with perfect certainty by means of the serum for which I propose the name*, "Antiphthisic Serum T. R."

It would be unnatural not to consider the possibilities that my serum may hold out for the treatment of human tuberculosis, although it is with great reluctance that I venture to make a few remarks on this point. After what has been said in the beginning of this paper, I take the applicability of antitoxin treatment in human tuberculosis for granted. Moreover, the chronic form in which this process is usually met with in man has certainly to be considered as a favorable point. It can be shown experimentally that the relative toxicity of the watery extracts of finely ground tissus of the organs of tuberculous guinea pigs is enormously higher than that of human tuberculous tissues; in other words, this very chronicity is

([23]). Toxine et antitoxine cholérique, Ann. de l'Institut Pasteur, X, 1896.

indicative of a process less productive of toxins. As an admissible objection, it might be said that one is not allowed to infer from phenomena observed in one animal, to those observed in another. The more penetrating our investigations become, the more we are forced to admit that the virulence and toxicity of a micro-organism is by no means the same upon different animals. I think, however, that in the case of the tubercle bacillus, though for obvious reasons a direct proof cannot be had, we are perfectly safe in surmising that these differences, if existing at all, are only differences of degree, and very slight ones, too. In the multifariously confirmed transmissions from animal to man, and *vice versa*, I am inclined to see a confirmation of this surmise.

The antitoxic potency of my serum seems as yet, when compared with other antitoxic sera, to be small, but I don't know whether we have a right to doubt *a priori* its efficacy in man on that account; in the first place, we do not as yet know anything with certainty about the way in which this antitoxic property exerts itself, and whether we are allowed to estimate it quantitatively. (24)

But besides this it is a fact that the main toxic action is not exerted by the living bacilli, but by the dead and disintegrating ones, so that a smaller amount of antitoxic power supplied continually will be likely to meet all exigencies. These, however, are problems to be solved in the future. I will only repeat that there seem to be theoretically no limits to the degree of the antitoxic potency, and that practically it is a matter of time, and—since T. R. is a rather expensive article—of financial considerations.

The value of T. R. serum for human patients can

(24). See Berlin. Klin. Wochenschr. June 21, 1897, p. 551.

only be ascertained by a prolonged observation. In about twenty cases so far treated by me and several physicians in and outside of St. Louis, the results have been exceedingly gratifying. Of course I need not tax your patience by telling you what kind of cases we may reasonably expect to be benefited by such treatment. I must lead your attention, however, to the statements of *Spengler* ([25]), asserting that a great number of so-called "mixed infections" are not *a priori* to be considered as hopeless, but that the secondary infection very often rapidly subsides and disappears as soon as some curative influence comes to bear on the tuberculous process.

All of the cases treated, so far as the reports show, were early cases of pulmonary affection; a positive diagnosis was made in every one of them by microscopic examination. In all of these cases within six to eight weeks a very decided improvement was brought about; temperature became perfectly normal; cough, expectoration, and night sweats stopped; uniformly a considerable increase of weight was observed; the pulse became normal, number of respirations decreased, etc.; in all cases physical examination showed an arrest of the active process and a clearing up of the affected area; in those cases observed by me the moist rales disappeared within four weeks after treatment began. The latter consisted in daily hypodermic injections of 1 cc. of the serum. No local or general reaction resulted, except now and then a little soreness and swelling around the site of the injection. The most noticeable fact was the lowering of the afternoon temperature, which sometimes would be observed after the first few injections.

(25). Zeitschr. f. Hyg. u. Infect. Krankheiten XVIII, 1897, Ueber Tuberculose und bei ihr vorkommende secundaere Infectionen.

I would not, however, like to lay myself open to the reproach of hasty conclusions in a subject the chief element of which is time. A more extensive report will be rendered after the necessary time has elapsed.

The scientific gain of my investigations is the preparation of a really antitoxic and bactericidal antiphthisic serum. With a probability next to certainty we may expect this serum to become an important factor in the preventive and curative treatment of human tuberculosis. The importance of the declining attitude of the Moscow Congress towards serum treatment in tuberculosis will, I am sure, dwindle down to the insignificance and worthlessness inherent to all judgments of gregarious masses.

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TABLE I.

Influence of T. R. Serum on the Temperature of Healthy Guinea Pigs.

Date.	No. of G. Pig.	Weight in grammes.	Amount of Serum injected.	Temper. before injection.	Hours Later.			
					3	6	9	12
VII-16	52	750	0.25 cc.	101.6	100.8	101.0	101.2	101.5
VII-16	53	640	0.50 cc.	102.1	101.0	101.5	101.8	102.2
VII-16	54	680	1.0 cc.	101.8	101.2	101.2	101.4	101.6
VII-16	56	706	2.0 cc.	101.7	100.6	100.8	101.6	101.6

TABLE II.

Effect of Normal Horse-Serum on the Temperature of Healty Guinea Pigs.

Date.	No. of G. Pig.	Weight in grammes.	Amount of Serum injected.	Temper. before injection.	Hours Later.			
					3	6	9	12
VII-17	55	714	0.50 cc.	102.2	101.6	101.6	102.0	102.4
VII-17	57	685	1.0 cc.	102.7	101.3	101.6	102.7	102.2

TABLE III.

Guinea Pigs After Previous Immunization with T. R. Serum Inoculated with a Fatal Dose of Tubercle Bacilli.

No. of G. Pig.	Weight July 5.	Received 4 cc. of Serum in 0.25 cc. doses fr'm VII-5 to VII-3.	Weight Aug. 3.	Inoculated with fatal dose of T. R. VIII-3.	Weight Sept. 20.	Remarks.
17	850	"	867	Subcute.	880	Sept. 20, perfectly healthy.
20	740	"	748	"	764	Sept. 20, perfectly healhy.
21	874	"	883	Intraperiton.	888	Killed IX-20. No lesions.
24	560	"	571	Not inoc.	588	Healthy.
25	674	"	692	" "	712	"
32	....	.....	721	Subcute.	....	Died VIII-27.
33	....	.....	856	"	....	Died VIII-24.

TABLE IV.

Immunization Experiment. 1.25 cc. Serum T. R.

No. of G. Pig.	Weight on VII-5.	Received 1.25 cc. in 5 injections from VII-5 to VIII-3.	Weight on VIII-3.	Fatal dose of Tuber Bac. on VIII-3.	Weight on IX-20.	Remarks.
18	716	"	724	Inocula'd	723	Extensive
19	635	"	638	"	636	Infiltrat'n
27	560	"	575	"	.....	Died VIII-23
58	612	"	621	Not Inoc.	629	.....
59	457	"	468	"	476	.....

TABLE V.

Immunization Experiment. 2.5 cc. of Serum.

No. of G. Pig.	Weight on VIII-5.	Received 2.5 cc. in 5 injections from VII-5 to VIII-3.	Weight on VIII-3.	Fatal dose of Tuber Bac. on VIII-3.	Weight on IX-20.	Remarks.
60	672	"	675	Inoc. VIII-3	677	
61	457	"	471	"	475	Perfectly
62	489	"	496	"	501	
63	723	"	729	Not Inoc.	738	Healthy.
64	594	"	617	"	629	

TABLE VI.

Serum and Virus injected mixed.

No. of G. Pig.	Weight VII-14.	Mode of Inoculation.	Weight IX-16.	Remarks.
1	456	T. B. + ½ cc. Ser. Subcut.	469	Healthy
2	567	T. B. + ½ cc. "	581	"
3	496	T. B. + ½ cc. "	517	"
37	712	T. B. + ½ cc. Serum Intraperit.	730	"
38	591	T. B. + ½ cc. "	612	"
39	623	T. B. + ½ cc. "	627	"
65	720	T. B. + 1 cc. Ser. Subcut.	731	"
66	635	T. B. + 1 cc. "	649	"
67	587	T. B. + 1 cc. "	610	"
68	421	T. B. + 0.25 cc. "	437	"
69	566	T. B. + 0.25 cc. "	575	"
70	578	T. B. + 0.25 cc. "	592	"
71	703	T. B. + 0.1 cc. "	....	Died, VIII-20
72	627	T. B. + 0.1 cc. "	534	Large Infiltrat'n
73	651	T. B. + 0.1 cc. "	....	Died, IX-6
31	561	Fatal dose of T.B alone	....	" VIII-7
32	622	" "	....	" VIII-3
33	786	" "	....	" VIII-12
34	612	T. B. + 0.25 cc Paquin's Ser. Subcut.	....	" VIII-5
35	659	T. B. + 0.25 cc "	....	" VIII-1
36	477	T. B. + 0.25 cc "	....	" VII-30

TABLE VII.

Serum and Virus Injected Separately.

No. of G. Pig.	Weight, VII-19.	Mode of Inoculation.	Weight, IX-12.	Remarks.
11	425	T. B. + 0.25 cc. serum.	439	Healthy.
12	553	"	576	Transitory glandular enlargement.
13	496	"	519	Healthy.

TABLE VIII.

Treatment Begun four Days after Inoculation.

No. of G. Pig.	Inocul'd with fatal dose of T. B.	Weight, VII-20.	Beginning of treatment, 0.25 cc. Serum every other day.	Weight, IX-27.	Remarks.
4	VII-20.	560	VII-24.	572	Perfectly healthy.
5	"	544	"	578	
6	"	489	"	500	
30	"	621	"	629	
40	"	576	"	587	
41	"	611	"	619	

TABLE IX.

Treatment Begun Seven Days after Inoculation.

No. of G Pig.	Inocul'd with fatal dose of T. B	Weight, VII-20.	Beginning of serum injections, 0.25 cc. every other day.	Weight, IX-24.	Remarks.
7	VII-20.	476	VII-27.	482	Healthy.
8	"	496	"	503	
9	"	521	"	536	
10	"	576	"	550	
11	"	488	"	499	
12	"	517	"	523	

TABLE X.

Treatment Begun Ten Days After Inoculation.

No. of G. Pig.	Inoc. with Fatal Dose of T. B.	Weight, VII-21.	Begin. of Serum Inj. 0.25 cc. Every Other Day	Weight IX-27.	Remarks.
13	VII-21	610	VII-31.	612	Healthy.
14	"	528	"	529	"
15	"	477	"	479	Swell. of Inguin. Glands
16	"	531	"	534	Healthy.
17	"	601	"	....	Killed IX-3.
18	"	576	"	....	"

TABLE Xa.

Treatment Begun Fourteen Days After Inoculation.

No. of G. Pig.	Inoc. with Fatal Dose of T. B.	Weight VII-21.	Treatm'nt with 0.25 cc. Every Other Day Began	Weight IX-27.	Remarks.
26	VII-21.	523	VIII-3.	456	Gland Swell. Ulcerat'n
28	"	614	"	....	Died IX-4.
29	"	539	"	474	All Signs of Tubercul's

TABLE Xb.

Controls. Not Treated.

No. of G. Pig.	Inoc. with Fatal Dose of T. B.	Weight VII-21.	Not Treated.	Weight IX-24.	Remarks.
42	VII-21.	507	"	.....	Died VIII-15
43	"	563	"	.....	" VIII-18
44	"	492	"	.....	" VIII-11

TABLE Xc.

Controls. Treated with Paquin's Serum.

No. of G. Pig.	Inoc. with fatal dose of T. B.	Weight VII-21.	Inj. of 0.25. cc. Paquin's Serum every other day began	Weight IX-27.	Remarks.
45	VII-21	516	VII-25	.....	Died VIII-13.
46	"	476	"	.....	" VIII-17.
47	"	567	"	.....	" VIII-10.

TABLE XI.

Showing Effect of Tuberculin Injection After Six Weeks of Treatment.

No. of Guinea Pig.	Date of Inj. of 0.1 cc. Tuberculin.	Temp. at Time of Injection.	Hours Later.					
			3	6	9	12	15	18
13	IX-12 10 a.m.	101.8	101.9	101.8	102.1	101.8	102.0	101.6
14	IX-12 2 p.m.	102.3	102.2	102.0	102.4	101.8	101.9	102.2
16	IX-14 8 a.m.	102.0	101.8	102.0	102.2	102.0	102.2	102.4

TABLE XII.

Showing Effect of Serum Treatment on Temperature. First Injection, VII. 31, at 12 a. m.

No. of G. Pig.	VII-29 8 a.m.	VII-29 8 p.m.	VII-31 8 a.m.	VII-31 8 p.m.	VIII-1 8 a.m.	VIII-1 8 p.m.	VIII-3 8 a.m.	VIII-3 8 p.m.	VIII-5 8 a.m.
13	103.6	104.4	104.0	103.6	103 0	102.6	101.8	102.2	101.6
14	104.2	104.8	103.8	103.0	102.2	102.6	102.0	102 4	102.0
16	104.2	105.2	104.8	104.0	103.2	101.8	102.4	102.0	102.2

TABLE XIII.

Test for Bactericidal Property of the Serum.

Contact between T. Bac. and Serum lasted hours.	1 cc. of T. B.-Serum mixture injected intraperitoneally into G. Pig No.	Date of Injection.	Weight IX-20.	Remarks.
1	48 (weight 520)	VIII-5	....	Died VIII-21.
2	49 (" 486)	"	....	" VIII-17.
3	50 (" 573)	"	....	" VIII-20.
5	51 (" 507)	"	516	No signs of disease.
8	74 (" 495)	"	519	Healthy.
12	75 (" 475)	"	489	"
24	76 (" 503)	"	517	"

TABLE XIV.

Inhibition of Tuberculin Reaction by Means of 0.1 cc. Serum.

Number of Guinea Pig.	Injected with	Temp. before Injec.	Temperature Hours Later.					
			3	6	9	12	15	18
74—Tuberculous for two weeks	0.1 Tuberculin +0.1 Serum.	103.9	103.8	104.0	103.6	103.8	104.1	103.9
75—Tuberculous for three weeks	"	102.4	102.6	102.4	102.8	102.2	102 6	102.4
76—Tuberculous for ten days	"	103.2	103.4	103.4	103 2	103.6	103.0	103.0
77—Tuberculous for two weeks	0.1 Tuberculin	103.0	103.0	103.6	104.2	104.8	104.6	103.8

TABLE XV.

Repeated Toxin Reaction in the Same Animal.

Number of Guinea Pig.	Injected with	Date of Injection.	Temp. before Injection.	Temperature Hours Later.					
				3	6	9	12	15	18
78—Tuberc. for about twenty days	1 cc. Toxin.	IX-1	102.8	103.8	104.2	104.3	104.6	104.2	103.8
79—Tuberc. for sixteen days	"	"	103.2	104.4	105 0	104.8	104.8	104.2	103.6
78	"	IX-4	103 0	104.6	105.4	105.0	105.2	104.2	103.8
79	"	"	102.6	103.8	104.6	104.4	104 4	104.0	103.6
78	"	IX-8	103.7	104.8	105.6	105.2	105.4	104.8	104.0
79	"	"	102.8	104 2	104.4	104.8	105.0	104.6	104.0
78	"	IX-11	102.6	104.0	104.6	104 4	104.0	104.2	103.6
79	"	"	103.4	104.4	104.8	105 0	105.0	104.2	103 6
78	1.5 cc. Toxin.	IX-14	102.4	104.8	104.8	105.0	104.6	104.4	103.6
79	"	"	103 2	104.2	104 6	104.8	104.2	104.0	103.0
78	"	IX-17	103.0	103 8	104.2	104 8	104.6	104 6	104.0
79	"	"	102.4	103 6	103.8	104.6	104.2	104.0	103.4