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SOME PRODUCTS OF THE TUBERCULOSIS BACILLUS AND THE TREATMENT OF EXPERIMENTAL TUBERCULOSIS WITH ANTITOXIC SERUM.*

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So much has been written in regard to the poisons of the tuberculosis bacillus that a review on this occasion would demand too much time, and we desire to refer only briefly to the work which is of importance in connection with those substances which we shall describe.

Tuberculin, as is well known, is the extract of the tuberculosis bacilli, including the media upon which they are grown. From specially prepared artificial cultures of the tuberculosis germ, Kühne (1) and the writer (2) (*Bulletin No. 7*, Bureau of Animal Industry) have obtained a substance corresponding to a nucleoalbumin, which appeared to be the fever-producing principle of the germ. However, many conditions in tuberculosis were not accounted for by this substance, and as Mafucci (3), Prudden and Hodenpyl (4), Vissman (5), and others had succeeded in producing tuberculous nodules without necrosis by the intravenous injection of dead

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either from cultures or from bodies of the bacilli themselves, some substance which might be considered accountable for the coagulation necrosis of tissue which takes place, a necrosis which it appears is necessary for the progress of the disease. This problem was undertaken by us more than two years ago. After many fruitless attempts we succeeded in isolating from artificial liquid cultures a crystalline substance having a melting point of 161° bacilli, it seemed as though it should be possible to isolate, to 164° C., readily soluble in ether, alcohol, and water, which separated from these solutions in needlelike or prismatic crystals showing a slight yellow tint (Plate I, Fig. 1). They did not give the biuret reaction. The solution of this substance has an acid reaction to litmus,

FIG. 1.

is acid in taste, and is optically inactive. The crystals give no precipitate with silver nitrate ($AgNO_3$), platinum chloride ($PtCl_4$), or barium hydrate ($Ba(OH)_2$). The analysis showed C = 50.88 per cent., H = 6.70 per cent., O = 42.42 per cent., giving a formula cor-

responding closely to $C_7H_{10}O_4$. This is the formula of teraconic acid, an unsaturated acid of the fatty series.

The culture medium upon which the bacilli were grown, and from which these crystals were obtained, contained potassium acid phosphate, ammonium phosphate, asparagin, and glycerin, the medium used and described by one of us (de Schweinitz) (6) some years ago for studying their products. After the growth on this media continues for some weeks the liquid becomes light yellow in color, having the appearance of a pale urine, a change which does not take place in the uninoculated medium kept under the same conditions. Efforts to obtain this same acid from the ordinary beef-broth cultures contain-

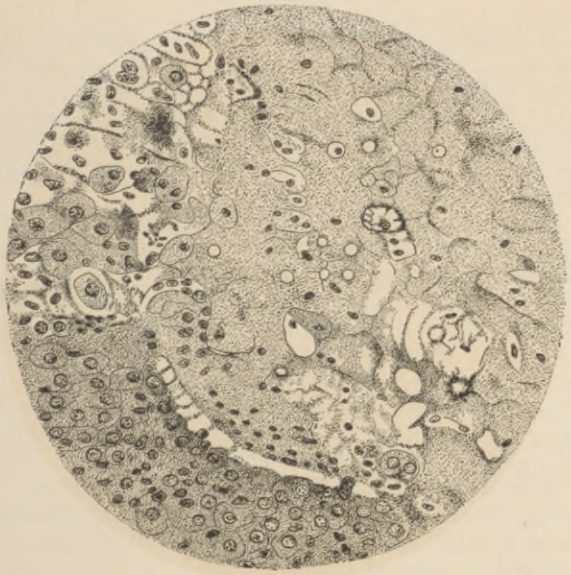

FIG. 2.

ing peptone and glycerin resulted in securing minute amounts of the crystals only, which it was never possible to purify. After noting some of the other properties of this acid substance we came to the conclusion that the presence of peptone and the nitrogenous bases of the

meat resulted in their combination with the crystals, forming compounds from which the acid could not again be easily extracted, even after the addition of acid. Finally, a small quantity of the crystalline substance obtained from the artificial cultures was added to the glycerin peptonized beef-broth medium, but it was impossible to recover it again by the methods used for the first extraction—viz., repeated precipitation with alcohol and extraction with water and ether. The ready solubility of this substance in water, as well as ether, probably accounts for the difficulty of obtaining it. The uninoculated medium did not yield these crystals. When dissolved in water and injected into guinea-pigs the following effects were noted:

I. *Healthy Guinea-pigs.*—No. 314. Received 0.015 gramme crystals. Temperature at time of injection, 102.6° F.; temperature at 11.50 A. M. (one hour after), 100.6° F.; temperature at 1.30 P. M., 100.2° F.; temperature at 3 P. M., 102.4° F.; temperature at 4 P. M., 102.2° F. During the above period the breathing was rapid, with an occasional rigor.

Guinea-pig No. 422. Weight, 284 grammes. Received 0.0095 gramme of crystals at 12.05 P. M. Temperature at time of injection, 99.8° F.; temperature at 2.30 P. M., 97.4° F. On the next day there was quite a perceptible swelling where the injection was made. The pig was chloroformed at the end of twenty-four hours and showed considerable inflammation at the seat of injection. The tissues were hæmorrhagic and bathed in a serous exudate. The muscular tissue was much disintegrated, resembling the appearance from the action of a caustic.

Guinea-pig No. 511. Weight, 183 grammes. Received, 0.0048 gramme in half a cubic centimetre of water at 11.25 A. M., subcutaneously in thigh. Temperature at time of injection, 103° F.; temperature at 12.25 P. M. (one hour after), 101.8° F.; temperature at 1.15 P. M., 102° F.; temperature at 3.25 P. M., 100.8° F. Chloroformed next day. Considerable inflammation, with serous exudate at seat of injection.

Guinea-pig No. 10. Received 0.0274 gramme at 10.10 A. M. Temperature at time of injection, 99.2° F.; temperature at 10.40 A. M., 100.2° F.; temperature at 11.15 A. M., 100.6° F.; temperature at 11.50 A. M., 100.6° F.; temperature at 2 P. M., 100.2° F. During above period

this pig showed signs of restlessness, breathed rapidly, and shivered.

II. *Tuberculous Guinea-pigs.*—Guinea-pig No. 181. Received 0.017 gramme at 10.25 A. M. Temperature at time of injection, 102.6° F.; temperature at 11.40 A. M., 101.4° F.; temperature at 1.50 P. M., 102.8° F.; temperature at 2.50 P. M., 103.4°; temperature at 3.50 P. M., 103° F. Pig sat drawn up in cage and shivered.

Guinea-pig No. 259 had received virulent tuberculosis two weeks previous to injection of crystals. Received 0.0172 gramme at 10.45 A. M. Temperature at time of injection, 102.4° F.; temperature at 11.45 A. M., 101.6° F.; temperature at 3.20 P. M., 101.6° F. Distinct rigors and rapid breathing.

Guinea-pig No. 377, inoculated with sputum from a tuberculous patient some weeks before the injection of crystals. Received 0.023 gramme at 11.35 A. M. Temperature at time of injection, 101.2° F.; temperature at 11.35 A. M., 100.6° F. Trembling very noticeable.

Guinea-pig No. 11 had received attenuated and virulent tuberculosis culture some time before (weight, 448 grammes). Received 0.0096 gramme at 11.25 A. M. Temperature at time of injection, 103° F.; temperature at 12.25 P. M., 100.8° F.; temperature at 1.15 P. M., 101° F.; temperature at 3.25 P. M., 100.8 F.

The idea was suggested from these experiments that this acid, evidently a secretion of the germ, was one of its most powerful weapons, that by its action upon the tissue the cells were first destroyed so that they could subsequently be utilized by the germ as food, and in this way the germ protected itself from surrounding leucocytes. To test this, crystals dissolved in sterile water were injected by means of a hypodermic syringe directly into the liver tissue. At the same time the same quantity of water was injected into a check in the same way. After forty-eight hours the check and experimental animals were killed. The check failed to show any effect, while the other exhibited a liver with several light spots. A repetition of this experiment gave the same results.

No effort was made to recover these crystals from the liver as the amount used was too small. We did not test the effect upon the liver by an intravenous injection, as would otherwise have been done, because we had

found that there was a combination of this acid substance with the albuminoids or bases, and any intravenous injection would have resulted in its immediate conversion into a modification by uniting with the albuminoids in the blood. Further, the growth of the germ in the body is localized, and where localized the necrotic areas are apparent, so that the fairest test was to bring the substance as soon as possible in contact with the tissue. The experiments in injections of the animals and appearance of sections follow:

Injection of Crystals from Artificial Cultures of Bacillus Tuberculosis into the Liver of Guinea-pigs.— Guinea-pig No. 409. Received 0.00178 gramme in liver on left side at 11.45 A. M. Temperature at time of injection, 101.6° F.; temperature at 3 P. M., 102° F. Chloroformed October 24, 1896, at 12 M. Liver dark, showing one or two small white spots and apparently a small inflamed spot at the point where the injection was made. Gall bladder was injected and seemingly inflamed.

Guinea-pig No. 412. Received 0.0037 gramme in liver at 1.45 P. M. Temperature at 1.45 P. M., 103° F.; temperature at 3.30 P. M., 100.4° F. Chloroformed at end of forty-eight hours. Gall bladder congested (not so much as in No. 409). Liver pale in spots, and one or two small white areas of apparent necrosis. The liver, hardened in $HgCl_2$, showed on microscopic examination one area rather well defined where the liver cells did not take hæmatoxylon well, though nuclei stain slightly.

Guinea-pig "C" received 0.0043 gramme crystals in liver. Chloroformed after six days. Pig weighed six hundred grammes.

Lungs were very slightly congested. In large left lobe of liver there were two or three comparatively large areas of necrosis. These spots were on the side in which injection was made, and the liver appeared to show the track of the needle. The guinea-pig was otherwise healthy.

Guinea-pig "E" received 0.0023 gramme of crystals in liver. Chloroformed after two days. Pig weighed three hundred and forty-five grammes.

All organs appeared normal, except the stomach, which showed a slight inflammation in its wall on the side which lay next to a necrosed spot in the liver. Besides this spot there were several others of considerable

size in the liver on the side on which the injection was made. The section of pig "C," the one allowed to live six days after injection, showed, on microscopic examination, the following:

Stained with hæmatoxylon and eosin, distinct areas of necrosis were noted, the most marked ones near the surface of the liver. Polynuclear leucocytes are present, though not in large numbers, in and around the necrotic areas, and there was also an increase in the connective-tissue cells of the liver around these same areas. Plate I, Fig. 2, is a drawing of the liver section showing healthy and necrosed areas.

Checks on Injection of Crystals into Liver.—Guinea-pig No. 510. Received a fourth cubic centimetre sterile distilled water in liver. Chloroformed after forty-eight hours. Post-mortem showed all organs—liver, lungs, spleen, etc.—normal.

Guinea-pig No. 387. Received half a cubic centimetre of sterile distilled water in liver. Chloroformed after forty-eight hours. Post-mortem: All organs were normal, excepting one or two very small pale spots in the liver; no necrosis.

Prudden (7), 1892, suggests that caseation, so constantly present in tuberculosis, is probably due to a specific metabolic product of the bacillus.

It seems very reasonable to conclude from our experiments that we have here the substance formed by the bacillus which is responsible for the coagulation necrosis.

The formula which can be deduced from the analysis makes this acid correspond closely to teraconic, which has properties very similar to those noted by us in connection with this new acid. Its identity we have not yet proved or disproved, as the preparation of teraconic acid is not completed. The amount of acid obtained is very small, so that we have used only a very minute portion of it for testing its immunizing property. A single injection of 0.0020 gramme was sufficient to keep the animals alive some weeks longer than the checks, and its solution appeared to exert some slight bactericidal influence.

As this substance seemed to be a temperature-reducing principle in healthy and diseased animals, we en-

deavored to separate the fever-producing principle independently. The crystals were always found in the culture liquid, and only minute amounts could be obtained for the bacilli themselves that had been grown on liquid medium. Accordingly, these bacilli, carefully filtered without heat, were washed in cold water, and next extracted with hot water. This hot water extract contained an albuminoid which caused the tuberculin reaction in guinea-pigs and calves upon repeated injections.

Roux and Nocard (8) state that they have a tuberculin which will give reactions almost indefinitely, but do not describe its method of preparation. Whether this is the same substance that we have obtained I am unable to say, but certain it is that the tuberculin prepared in the way we have indicated will give reactions four or five times in succession, where the reaction with tuberculin as prepared in the ordinary way fails after the second time. The conclusion is a fair one, I think, that the fever-reducing principle having been removed, to an extent, if not entirely, the immunity to the fever-producing principle is much more slowly acquired. Our tests upon guinea-pigs and tuberculous calves were made with only one day intervening between the injections (see Table I).

In the *Deutsche medicinische Wochenschrift* for April 1, 1897, Dr. R. Koch (9) describes some new tuberculin preparations. The dried tuberculosis bacilli were taken (the culture medium used is not mentioned), finely powdered and centrifugalized with distilled water. The opalescent solution obtained, tested upon animals, gave the tuberculin reaction. The residual germs were submitted to this treatment a number of times, until finally all were practically dissolved. The latter solutions in large doses caused a reaction, but in small quantities did not produce this result, and seemed to exert both an immunizing and curative action in experimental tuberculosis. Koch used for this work virulent germs, and claims that attenuated germs do not give an active product. My own work was done with bacilli purposely attenuated by cultivation, and the results show that very active fever-

TABLE I.—*Tests of Cell Extract, Tuberculin, and Serum.*

Date.	No. of animal.	Condition.	Weight in grammes.	Substance injected.	TEMPERATURE.		
					11.20 A. M.	1.40 P. M.	3.05 P. M.
March 26....	XI	Tuberculous guinea pig.	423	¼ c. c. tuberculin + ½ c. c. serum.	103·2°	96·6°	96·2°
	492	Tuberculous guinea-pig.	255	¼ " tuberculin.	102·8	105·0	104·4
	VIII	Attenuated tuberculosis.	443	¼ " tuberculin + ½ c. c. serum.	101·0	102·4	103·8
	XIX	Attenuated tuberculosis.	356	2 " cell extract = 0·0040 gramme.	102·2	105·6	103·4
	513	Healthy (check).	210	1 " cell extract = 0·0020 gramme.	101·4	101·6	101·1
					10.50 A. M.	12.25 P. M.	1.50 P. M.
March 27....	XI	Tuberculous guinea-pig.	...	¼ c. c. tuberculin.	101·2°	102·4°	102·4°
	492	Tuberculous guinea-pig.	...	2 " cell extract = ¼ c. c. tuberculin.	103·0	103·8	105·0
	VIII	Attenuated tuberculosis.	...	¼ " tuberculin.	103·0	103·2	103·8
	XIX	Attenuated tuberculosis.	...	¼ " tuberculin.	102·8	103·8	103·0
	XX	Attenuated tuberculosis.	...	¼ " tuberculin + ½ c. c. serum.	102·4	95·0	95·0
					10.25 A. M.	12.10 P. M.	1.45 P. M.
March 29....	VIII	Attenuated tuberculosis.	...	2 c. c. cell extract = ¼ c. c. tuberculin.	101·8°	103·8°	103·6°
	492	Tuberculous guinea-pig.	...	2 " cell extract = ¼ c. c. tuberculin.	102·8	104·4	104·6
	XIX	Attenuated tuberculosis.	...	2 " cell extract = ¼ c. c. tuberculin.	102·2	105·6	103·0
					10.45 A. M.	12.15 P. M.	2.10 P. M.
March 30....	492	Tuberculous guinea-pig.	...	2 c. c. cell extract = ¼ c. c. tuberculin.	102·2°	104·2°	104·2°
	XIX	Attenuated tuberculosis.	...	¼ " tuberculin.	102·2	103·2	103·6

producing, fever-reducing, and probably curative principles can be obtained from them. It hardly seemed justifiable to myself or others to powder dried virulent bacilli and have the dust floating in the air. Koch further refers to two fatty acids which, in conjunction with Proskauer (9), had been found in the bodies of the germs. The writers (10) of this paper published in the *American Chemical Journal*, August, 1895, a preliminary study of the fats of the tuberculosis bacilli, showing the high content of fat in the bodies of these germs, which accounts for the difficulty in staining them with certain colors, as well as their difficult absorption.

In a later paper, *Centralblatt für Bak. u. Parasitenkunde*, 1896 (11), we described briefly the different acids obtained from the body of the germ, both high melting and low melting acids, but whether or not these are identical with those observed by Koch and Proskauer we can not tell from the brief mention made of them.

From our results it seems very reasonable to think that the necrotic acid is the fever-reducing principle, the albuminoid the fever-producing principle, and the reason the tuberculin ordinarily does not react continuously is on account of their presence at the same time. At any rate, tuberculous guinea-pigs tested successively with tuberculin showed no reaction, while with this albuminoid, which we will call cell extract, a reaction was obtained.

The preliminary experiments, published by one of us in 1894 (12), upon the production of an immunity or resistance to tuberculosis by attenuated cultures have been continued and are confirmatory of the first results, showing the production of great resistance and in some cases complete immunity. A detail of two sets of these experiments may be given as an instance of their general results (Table II).

The first effect of the injection of the attenuated germ was in some instances to cause a slight decrease in weight; sometimes a local swelling was noted at the point of injection, and occasionally an enlargement of the inguinal glands. This disappeared after some weeks. This

TABLE II.—*Two Sets of Experiments showing the Average of Results in Experiment in which the Guinea-pigs were Vaccinated with Attenuated Germ and then Inoculated with Virulent Germ.*

No.	Date of inoculation and amount of attenuated germ.	Weight, Oct. 24.	Weight, Nov. 2.	Condition.	Date of inoculation and amount of virulent germ.	WEIGHTS. Dec. 16.	Jan. 7.	Jan. 19.	Feb. 2.	Feb. 8.	April 8.	April 19.
373	Oct. 24, 1½ c. c.	12 oz.	14 oz.	O. K.	December 9th: 373, 375,							
374	Fiftieth generation.	10 "	11 "	....	and 378 dead from pneu-	14 oz.	16 oz.	16 oz.	15½ oz.	16 oz.	16 oz.	14 oz.
375	Fiftieth generation.	16 "	15 "	O. K.	monia; 374, 376, and 377							
376	Fiftieth generation.	16 "	14½ "	O. K.	each received ⅛ c. c. of	12 "	15 "	15 "	15 "	15 "	17½ "	18 "
377	Check.	13 "	13¾ "	O. K.	vir. tuberculosis; 4th gen-	12 "	13 "	12½ "	Dead.			
378	1½ c. c. attenuated germ.	14 "	14 "	Thin.	eration from rabbit.							

No.	Dec. 21.	Dec. 26.	Dec. 21.	Feb. 2.	Feb. 12.	March 8.	March 16.	April 6.	April 19.	April 21.
II.	All but	All given	13 oz.	14 oz.	16 oz.	All, including checks (X	16 oz.	Chloroformed.		
III.	X and XI	1½ c. c. of	12 "	15½ "	14 "	and XI), inoculated with	17 "	17 oz.	19 oz.	
IV.	(checks)	61st gen-	15 "	16 "	17 "	⅛ c. c. virulent germ.	20 "	21 "	20 "	
V.	received	eration	13 "	15 "	16 "		17½ "	19 "	19 "	
VII.	½ c. c. of	except	14 "	13 "	14 "		16 "	16½ "	18 "	
VIII.	61st gen-	checks	15 "	15 "	16 "		17 "	16 "	18 "	
IX.	eration.	(X and XI).	12 "	13 "	11 "		14 "	15 "	16 "	
X.			.....	16 oz. ch.	16 oz. ch.	18 oz. } checks Mar. 6.	17½ oz. ch.	14 oz. ch.	12 oz. ch.	Dead ch.
XI.			.....	15½ oz. ch.	16 " "	17½ " }	17½ " "	Dead.		
XII.			16 oz.	16 oz.	16 oz.		18 oz.	19 oz.	19 oz.	
XIII.			14 "	15 "	16 "		18 "	17½ "	16½ "	
XV.			15 "	16 "	18 "		18 "	14 "		
XVI.			15 "	15 "	17 "		18 "	18½ "	20 oz.	
XVII.			12 "	12 "	13 "		15 "	15 "	14½ "	
XVIII.			15 "	15 "	16 "		16 "	17½ "	17½ "	
XIX.			14 "	13 "	14 "		13 "	13 "	12½ "	
XX.			15 "	18 "	18 "		20 "	19 "	19 "	

local swelling we consider to be due to the mechanical action of the body of the germ on account of their high fat content and possible presence of a minute amount of the acid causing necrosis. It does not always result from a subcutaneous inoculation, and an apparent immunity to this action is acquired by repeated injections. This is well shown in horses and cows submitted to treatment with the attenuated bacilli. From six to eight weeks after the date of the injection of the bacilli guinea-pigs seem to be entirely well, and are then inoculated with the virulent bacilli. As can be seen from the chart, the checks died within six weeks from date of inoculation, while the others vaccinated remained well four months afterward. It has appeared from the many experiments made that if the inoculation with the virulent bacillus is made before complete recovery from the treatment with the attenuated bacillus, the resistance is considerably less. The inoculation of the animals with the virulent bacillus, and subsequent treatment with a single injection of the attenuated germ, showed that the latter produced a slight resistance, but no very material retardation of the disease.

The production of this partial immunity or artificial resistance by means of the attenuated germ (12) suggested already in 1894 the availability of this same material for the purpose of treating animals for the production of a serum which would have some effect in curing tuberculosis. It suggested the idea, further, that possibly cattle could be vaccinated with this attenuated germ and made immune to tuberculosis.

Experiments tried in this latter direction will be reported later.

Two cows and one heifer were selected for the work, which was conducted for us by Dr. Schroeder, in charge of the Experiment Station of the Bureau of Animal Industry. One of these animals was originally tuberculous; the other two were healthy. To the tuberculous animal were given large doses of tuberculin until it had received altogether 19,407 cubic centimetres (nineteen litres and a half), and as much as 1,500 cubic centimetres of tubercu-

lin at a single dose from November, 1894, to April 20, 1897. The other animals received injections of the attenuated culture, the amount injected in fifteen months being 11,425 cubic centimetres and 18,100 cubic centimetres, respectively, and by this we mean the liquid culture *in toto*, including the bacilli thoroughly shaken in the media, forming an emulsion, just as taken from the incubator without any further treatment. At first the injections produced a slight reaction and occasionally a local œdema and abscess. After they had been continued for some time this effect diminished or disappeared. The serum of all of these animals was tested a number of times. Guinea-pigs were injected with the serum in quantities varying from one and a half to six cubic centimetres, and subsequently inoculated, together with the checks, with a tuberculosis bacillus sufficiently virulent to kill the checks within four or five weeks; or the pigs were inoculated with the virulent bacillus and treated by subsequent injections of the serum. Without giving the details of the experiments we may say that the serum from the cow treated with tuberculin would cause in the pigs a slight resistance to the virulent bacilli; the serum of the cows treated with the attenuated bacilli produced more resistance on the part of the guinea-pigs, or prolonged their life to some extent, but not sufficiently, as compared with the quantity of material injected, to make the use of cow serum appear practicable. The cow serum, although sterile, frequently produced abscesses in the guinea-pigs. This serum we expect to test again when it should be more active.

While these experiments were in progress two horses had been pressed into service. They were treated by injecting the attenuated cultures, culture fluid, and bacilli. The first injection of five cubic centimetres caused a decided temperature reaction, local œdema, stiffness, slight loss of appetite, recovery after a few days. At first local abscesses were formed, which healed fairly readily. After a time the abscess formation ceased. After eight months' treatment, the dose of the culture being gradually increased up to three to four hundred cubic centi-

metres at a time—the total amount injected in fifteen months being four thousand four hundred and fifty-nine cubic centimetres—the serum was used for testing. It separated out clear and well. Two sets of illustrations may be given to show its action on tuberculous animals. In one set (Table III) the checks and two treated pigs died; the other two treated pigs are alive, and in perfect health, apparently, after a number of months. In another set the checks, four in number, died within four to five weeks, while the treated ones lived two to three weeks longer, showing, on autopsy, much less disease in the lungs than the checks. We endeavored further to isolate from the serum antitoxic substances by a slight modification of the Brieger-Boer method. We finally succeeded in obtaining a small quantity of a grayish powder giving the biuret reaction, with difficulty soluble in water, which was used for treating guinea-pigs in the same way as the serum. The result was about the same as in the first instance. The pigs, half a pound in weight, were inoculated with a virulent germ and treated by a single injection of 0.008 gramme of this solid substance. They lived three or four weeks longer than the checks, the lungs again showing considerably less disease, and less necrosis was noted in the liver (Table IV).

The effect of the serum was also tried in preventing the rise of temperature in tuberculous guinea-pigs and in saving them from a fatal dose of tuberculin. As can be seen from the temperature reactions in Table I, the injections of one fourth of a cubic centimetre of diluted tuberculin, and at the same time of half a cubic centimetre of the serum, either caused a decided reduction of the temperature or prevented a characteristic tuberculin reaction in animals weighing five hundred grammes. This is one way of gauging the serum.

The result of all this work leads us to the conclusions that the injection of the live culture produces substances antitoxic to the disease which will cure tuberculous animals; that the quantity of this substance can be increased gradually; that the treatment of tuberculosis is and will be for some time still in the experimental stage. One point,

TABLE III.—*Serum from Horse Injected with Attenuated Culture used on Tuberculous Guinea-pigs.*

No.	Weight.	Date and amount of virulent culture.	DATES AND AMOUNT OF SERUM INJECTED.						
			Nov. 6.	Nov. 17.	Nov. 25.	Dec. 3.	Dec. 8.	April 19.	
434 ch.	10 oz.	Oct. 24th; $\frac{1}{4}$ c. c. of virulent tubercular culture given to all; all except check received $1\frac{1}{2}$ c. c. of serum.	10 oz.	$8\frac{1}{2}$ cz.	8 oz.	Dead.			
435	9 "		9 " $+1\frac{1}{2}$ c. c.	8 " $+1\frac{1}{2}$ c. c.	8 " $+1\frac{1}{2}$ c. c.	.....	9 oz.	20 oz.	Alive and well.
436	11 "		10 " $+1\frac{1}{2}$ "	9 " $+1\frac{1}{2}$ "	7 " $+1\frac{1}{2}$ "	Dead.			
437	14 "		13 " $+1\frac{1}{2}$ "	11 " $+1\frac{1}{2}$ "	10 " $+1\frac{1}{2}$ "	.....	10 "	20 "	Alive and well.
438	9 "		8 " $+1\frac{1}{2}$ "	8 " $+1\frac{1}{2}$ "	6 " $+1\frac{1}{2}$ "	Dead.			
439	8 "		8 " $+1\frac{1}{2}$ "	7 " $+1\frac{1}{2}$ "	5 " $+1\frac{1}{2}$ "				

TABLE IV.—*Tests of Dry Antitoxic Material from Serum from Vaccinated Horse.*

No.	Weight.	Date.	Material for inoculation.	Date.	Weight.	Date.		Date.	Weight.	Date.
464	Check, 11 oz.	Feb. 4.	$\frac{1}{8}$ c. c. virulent culture.	Feb. 20.	11 oz.	.....	.....	March 6.	10 oz.	March 8, dead.
476	12 "	" 4.	$\frac{1}{8}$ c. c. virulent culture + 0·008 grs. antitoxine.	" 20.	12 "	.....	.....	" 6.	10 "	March 16, dead; less disease than others.
478	Check, $8\frac{1}{2}$ "	" 4.	$\frac{1}{8}$ c. c. virulent germ.	" 20.	8 "	Feb. 26.	Dead; tuberculosis.			
479	Check, 8 "	" 20.	$\frac{1}{8}$ c. c. tuberc. virulent.	.....	.....	.....	.....	" 6.	7 "	March 8, dead; generalized tuberculosis.
481	Check, 10 "	" 20.	$\frac{1}{8}$ c. c. tuberc. virulent.	.....	.....	.....	.....	" 6.	9 "	March 12, dead.
482	13 "	" 20.	$\frac{1}{8}$ c. c. tuberc. virulent + 0·008 grs. antitoxine.	.....	.....	.....	.....	" 6.	$12\frac{1}{2}$ "	April 7, dead; less disease.
484	12 "	" 20.	$\frac{1}{8}$ c. c. tuberc. virulent + 0·008 grs. antitoxine.	.....	.....	.....	.....	" 6.	12 "	April 2, dead; less disease than others.

however, must be remembered—viz., that while it may be difficult to cure the disease in a guinea-pig, where its course is very rapid—a virulent bacillus requiring only from four to five weeks to kill—it might be much easier to check the disease when more prolonged in action, as in the majority of cases in man. Again, in addition to some form of specific treatment for the disease, man usually has the advantage of being placed under the best possible surroundings as to diet, climate, etc., and every effort is made to aid the improvement of the patient, while with experimental animals the conditions are different.

The experimental results obtained lead undoubtedly to the conclusion that while the treatment with antitoxic serum is still in the experimental stage and should be as yet only used in sanitariums and under the best conditions, we are on the road to success in the treatment of this disease and nearer our goal than ever before. In an experimental way the antitoxic serum as prepared in our laboratory has been used by Dr. Stubbert at the Loomis Sanitarium and some by Dr. Trudeau at Saranac Lake, as well as by Dr. C. W. Richardson in this city (Washington, D. C.).

Dr. Stubbert, out of six cases treated, reports one cured and others improved. Dr. C. W. Richardson notes decided improvement in his cases, while Dr. Trudeau, who has used some of the serum for a short time only, noted in one case a reduction of temperature which may have been due to this serum.

Maragliano, Babes, Behring, and Paquin are the other principal workers in the preparation of an antitoxic serum for tuberculosis.

Maragliano (13) gives the method he has used for the production of antitoxic serum, and notes that there is present in the cold filtered cultures of the tuberculosis bacilli, a substance which causes the reduction of temperature, and another not destroyed by heat, which causes the rise of temperature. In all probability, without isolating the principle, Maragliano was using solutions of the crystalline substance we have described in the beginning of this paper. While this is not destroyed by heat, as

he seems to think, it does undergo some change by combining probably with the albuminoid matter in the media, and thus losing its distinct property as a temperature-reducing substance. Or, more probably, its temperature-reducing property is disguised by the presence of the temperature-producing principle extracted by hot water. The serum which he claims to obtain from treatment of the animals is said to have some effect in reducing the temperature and apparently improving the disease.

Babes (14), reviewing a portion of the work upon the treatment of tuberculosis with serum, comes to the conclusion that he is the first individual to have discovered any antitoxic properties in the serum from treated animals; that there is an antitoxic substance present in this serum, but that it has not yet been brought to a sufficient development to warrant general use.

Our experiments lead us to conclude that while the injections with tuberculin produce a serum containing antitoxic material, the amount of this is small, and that the injection of the live culture is the proper treatment. We can not agree to the statements made that horses are unsuitable for the work. Mules and donkeys may perhaps give results more quickly, but horses seem to be eminently satisfactory. At no time have we found that the horse serum produces toxic effects, although this has been noted in the cow serum.

If the antitoxic serum treatment and other methods for tuberculosis could be freed for the present from their commercial aspect, and careful, systematic experiments continuously conducted in numerous hospitals and sanitariums, this or a similar modified method of treatment could be looked to for good results.

When tuberculosis can be uniformly cured in guinea-pigs as certainly as diphtheria, then does the commercial aspect become a fair and legitimate one.

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