BULLETIN No. 13.

U. S. DEPARTMENT OF AGRICULTURE.

BUREAU OF ANIMAL INDUSTRY.

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By E. A. de Schweinitz and Marion Dorset.

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By E. A. de Schweinitz.

Prepared under the direction of
Dr. D. E. SALMON,
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LETTER OF TRANSMITTAL.

U. S. DEPARTMENT OF AGRICULTURE,
BUREAU OF ANIMAL INDUSTRY,
Washington, D. C., July 15, 1896.

SIR: I have the honor to transmit herewith, and to recommend for publication as a bulletin of this Bureau, the manuscript containing a report of investigations upon the cultivation of the tuberculosis bacillus upon acid media, and of some further experiments with reference to the effect of tuberculin upon the milk of cows.

These experiments have been conducted under the immediate supervision of Dr. E. A. de Schweinitz, assisted by Drs. Marion Dorset and E. C. Schroeder, and the publication of the results obtained will furnish a convenient and desirable method of preserving and distributing the results of scientific investigations in this important part of the work of this Bureau.

Very respectfully,

D. E. SALMON,
Chief of Bureau.

Hon. J. STERLING MORTON,
Secretary of Agriculture.

LETTER OF SUBMITTAL.

U. S. Department of Agriculture,
Washington, D. C., July 1, 1896.

Sir: I herewith submit for publication some results of investigations upon the cultivation of the tuberculosis bacillus upon acid media, the innocuous character of an attenuated tuberculosis bacillus, and some further experiments with reference to the effect of tuberculin upon the milk of cows. Charts showing the average temperature reactions of cattle as regards breed are also appended.

Respectfully,

E. A. de Schweinitz,
Chief Biochemic Division.

Dr. D. E. Salmon,
Chief of Bureau of Animal Industry.

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TUBERCULOSIS INVESTIGATIONS.

THE GROWTH OF THE TUBERCULOSIS BACILLUS UPON ACID MEDIA.

By E. A. de Schweinitz, Ph. D., M. D., and Marion Dorset, M. D.

INTRODUCTION.

In the Philadelphia Medical News of December 8, 1894, one of us (De Schweinitz) published an article upon the attenuated bacillus tuberculosis and its effect upon guinea pigs, and called attention to the fact that the tuberculosis germ would grow satisfactorily upon media having an acid reaction. This appeared to be an important observation, and while we have for several years been cultivating the tuberculosis germ upon various media, some interesting facts have been noted which are worthy of record.

Pawlosky, Ann. de l'Institut Pasteur, 1888 (and Sander) (Archiv für Hyg., Bd. XVI, p. 238) have shown that vegetable broths may be used for the cultivation of the bacillus tuberculosis, and (in the Centralb. für Bak. und Parasit., August 8, 1895, Nos. 4, 5) Lubinski describes the utilization of this acid broth in the preparation of solid and liquid media, either with or without the addition of beef broth.

Our cultures have been made upon the ordinary peptonized meat broth prepared from fresh meat and upon the artificial media, which, as one of us noted earlier, has been very useful in studying the products of germs. We find that the tuberculosis bacillus will grow upon acid beef broth which requires 21.6 c. c. $\frac{N}{10}$ sodium hydrate solution to neutralize every 100 c. c. of the media. It will also grow upon acid artificial media every 100 c. c. of which requires 45 c. c. of $\frac{N}{10}$ sodium hydrate soltion for neutralization. This acidity in some instances was due to the acids in the meat. In the artificial media it was due to the acid phosphate of potassium used.

We have also added free hydrochloric acid to the acid beef broth and to the acid artificial media, so that from 1 c. c. to 3 c. c. $\frac{N}{2}$ hydrochloric acid have been contained in every 100 c. c. of the media. The presence of free hydrochloric acid was tested, both before and after the sterilization of the media, and after the bacilli had begun to multiply, by means of congo red or phloroglucin and vanillin. The congo-red test was not satisfactory in the beef broth, the albuminoids and possibly organic acids present evidently interfering with the reaction. The phloroglucin and vanillin test, however, proved conclusively the presence of free hydrochloric acid in the media, upon which there was a good growth of the tuberculosis bacillus. The exact quantity of the free hydrochloric acid present in these cultures could not be readily determined, as when hydrochloric acid was added to the media a portion apparently first decomposed the organic salts and phosphates.

After the growth of the tuberculosis germ is well advanced upon the media described and has apparently ceased, the acid reaction of the cultures is still more marked, requiring for the neutralization of the acid substance produced by the germ to every 100 c. c. of culture 12.5 c. c. to 15 c. c. $\frac{N}{10}$ sodium hydrate.

Lubinski notes a decided variation in the morphology of the germ as grown upon the acid vegetable broth. The bacilli, he says, often appear in long filaments, having, however, the same thickness that the germs usually possess. The filament forms described by Metschnikoff, Fischel, and Jones varied in thickness, showed side chains, and were not joined, while Lubinski reports characteristic streptococci-like formation. Jones could find this filamentous growth only after the cultures were four to five months old. Lubinski observed it in ten days.

OBSERVATIONS.

Our observations upon the character of the germs grown upon acid beef broth media and their morphological changes are somewhat different. Instead of a thread-like growth or chain growth we have often found what appeared to be spores, and sometimes the bacilli seem very much thicker and longer than the ordinary germs. In one culture examined the germ had been from generation to generation during four years grown on acid media and might be expected to show Lubinski's chain-like formation, but instead there was an apparent spore formation perceptible.

The appearance of the germs grown upon our acid media can be seen from the accompanying plates. Pl. I, fig. 1, shows the bacillus grown upon glycerin beef broth for four months; Neisser's spore stain. Pl. I, fig. 2, shows the bacillus grown for forty generations upon an acid media.

The filament forms are not observed. There is a slight thickening of the bacillus and rounding at the end often noted, which would appear to be spore formation. There is a different change in the morphology of the germ as grown upon our media which confirms the conclusions of Lubinski that we have heteromorphic forms of the tuberculosis bacillus and a genuine pleomorphismus caused by the changed conditions of life. Bruns (Centralblatt für Bak. und Parasitenkunde, Bd. XVII, Abth. I, No. 23) describes a germ which gives Neisser's spore reaction and in that particular evidently corresponds to the ones we have pictured.

FIG. 1.—Seventh generation in glycerine beef broth. Neisser's spore stain. (× 2000.)

FIG. 2.—Fortieth generation, acid media. Neisser's spore stain. (× 2000.)

Haines, del.

BACILLUS TUBERCULOSIS UPON NEUTRAL AND ACID MEDIA.

FIG. 1.—Culture containing free hydrochloric acid. Neisser's spore stain. (×2000.)

FIG. 2.—Artificial culture. Neisser's spore stain. (× 2000.)

Haines, del.

BACILLUS TUBERCULOSIS UPON ACID MEDIA CONTAINING FREE HYDROCHLORIC ACID, AND UPON ARTIFICIAL MEDIA.

Pl. II, fig. 1, shows the germ grown upon acid media containing free hydrochloric acid.

Pl. II, fig. 2, is a drawing of the tuberculosis germ grown on artificial media. We do not here observe quite the same change in the morphology of the germ.

DETAILS OF EXPERIMENTS.

A more detailed description of our experiments may be interesting. The reaction of the media prepared for the cultivation of the tuberculosis germ was not changed in the process of sterilization. A quantitative check of the amount of acid was made before and after this process.

First experiment.—The media used for this experiment was alkaline and required for neutralization 1 c. c. $\frac{N}{2}$, hydrochloric acid for every 50 c. c. of the media.

a. To each of 2 flasks 50 c. c. each, 5 c. c. $\frac{N}{10}$, NaOH were added.

b. To each of 2 flasks 50 c. c. each, 20 c. c. $\frac{N}{10}$, NaOH were added.

c. To each of 2 flasks 50 c. c. each, 1 c. c. $\frac{N}{2}$, HCl were added.

The six were inoculated at the same time with tuberculosis bacilli from (A 14). Within two weeks a good growth of the germ was noted over the surface of flasks *a* and *c*, while *b* showed no growth at all.

Second experiment.—The media used required for neutralization 8.4 c. c. $\frac{N}{10}$, sodium hydrate solution for every 50 c. c.

a. To each of 2 flasks 50 c. c. were added 8.4 c. c. $\frac{N}{10}$, NaOH.

b. To each of 2 flasks 50 c. c. were added 1.3 c. c. $\frac{N}{2}$, HCl.

a flasks were neutral and *b* flasks acid. The two sets were inoculated from the same culture (A 15). Within two weeks the germs in both flasks were growing well, *a* perhaps a trifle better than *b*. At the end of seven weeks one flask, *a*, was titrated and required for neutralization 6.3 c. c. $\frac{N}{10}$, NaOH. This flask had been neutral when inoculated. *b* flasks required 22.5 c. c. $\frac{N}{10}$, NaOH for neutralization. The increase in acid in *a* flasks is represented, therefore, by 6.3 c. c. $\frac{N}{10}$, NaOH, and in the *b* flasks by 7.5 c. c. $\frac{N}{10}$, NaOH. There was apparently a larger amount of the acid substance formed in the acid flasks than in those which were neutral at the time of inoculation.

Third experiment.—The media used required for neutralization 6.6 c. c. $\frac{N}{10}$, NaOH for every 100 c. c. of liquid.

a. To each of 2 flasks 100 c. c., 1 c. c. $\frac{N}{2}$, HCl was added.

b. To each of 2 flasks 100 c. c., 2 c. c. $\frac{N}{2}$, HCl were added.

c. To each of 2 flasks 100 c. c., 3 c. c. $\frac{N}{2}$, HCl were added.

These flasks were all inoculated with a virulent germ, and although it required several weeks for the growth to begin, after four weeks there was growth in all of the cultures, the most acid ones seeming to contain as good a growth as the others. Every 100 c. c. of the *a* media had required 11.6 c. c. $\frac{N}{10}$, NaOH.

Every 100 c. c. of the *b* media had required 16.6 c. c. $\frac{N}{10}$, NaOH.

Every 100 c. c. of the *c* media had required 21.6 c. c. $\frac{N}{10}$, NaOH for neutralization.

Fourth experiment.—The media used in this experiment was the artificial media described by one of us—De Schweinitz—in 1892, and it required 45 c. c. $\frac{N}{10}$, NaOH for every 100 c. c. for neutralization.

a 100 c. c. one flask of the media as noted.

b 100 c. c. one flask of the media as noted with the addition of 2 c. c. $\frac{N}{2}$, hydrochloric acid.

These were inoculated from another artificial culture, and both within fifteen days showed a good growth, the one containing hydrochloric acid showing a slightly better growth than the other.

Drs. Trudeau and Baldwin have pointed out that the apparent retardation of the growth of the tuberculosis bacillus by the acid reaction of the media can be overcome by neutralization. This is true; but we have also found that if the germ is transferred from an acid culture after it has been cultivated on acid media for a number of months to a neutral nutrient fluid it will no longer grow. There would seem from this to be probably a poisonous principle secreted by the germ. It may be a true acid or, more likely, a substance with an acid reaction. In the experiment, as the germs used for inoculation still floated on the surface of the culture they should have been alive unless some substance especially inimical to their growth was produced in the cultures.

These experiments show clearly that the tuberculosis bacillus can be readily accustomed to an acid nutrient fluid, that it can easily adapt itself to the changed conditions of life, and that even a small amount of free hydrochloric acid does not interfere with its growth. They tend to show further, we think, that under certain conditions there is probably a poisonous substance produced by the germs which is inimical to their own life.

The adaptability of the bacillus to a variety of media can best be explained by spore formation.

MARCH 14, 1896.

FURTHER EXPERIMENTS WITH AN ATTENUATED TUBERCULOSIS BACILLUS.

By E. A. DE SCHWEINITZ, Ph. D., M. D., and E. C. SCHROEDER, M. D. V.

In the Medical News, December 8, 1894, one of us (E. A. de Schweinitz) briefly described an attenuated tuberculosis bacillus (human), and noted that while originally very virulent, by a special method of cultivation it had been rendered very harmless, and not only failed to produce disease in guinea pigs and rabbits, but in some instances imparted to animals inoculated with it a distinct resistance to tuberculosis upon a subsequent inoculation with a virulent germ. The experiments with this attenuated germ have been continued, and we desire to present further evidence of the apparent absolutely harmless character of this attenuated germ, reserving for a later article further reports of its immunizing properties.

EXPERIMENT WITH A MONKEY.

After a number of guinea pigs and cattle had been inoculated with this attenuated germ it seemed desirable to test its effect upon an animal susceptible to tuberculosis and more like man. Accordingly a monkey placed at our disposal by Dr. Kinyoun was inoculated on September 10, 1895, with $\frac{1}{4}$ c. c. of an emulsion of a tubercle culture thirtieth generation. The inoculation was made from a culture in which there was an active growth.

On September 28, 1895, the injection with this material $\frac{1}{2}$ c. c. of tuberculosis culture thirty-second generation was repeated, and again on January 20, 1896, 2 c. c. of tubercle culture thirty-third generation. At the seat of the first and second injections small nodules were formed, while no local lesion was noted at the seat of the third injection.

On April 9 the monkey was found dead. It had not been well for three weeks and was quite sick during the five or six days immediately preceding death. It had no appetite, the hair was rough, there was nausea and great thirst and evidence of distress. Post-mortem examination revealed congested lungs and an inflammatory condition of ileum and caecum. Dr. Schroeder says "there were no signs of tuberculosis and the small nodules formed at the time of the first two injections had become absorbed." This attenuated germ was, therefore, innocuous to the monkey, though, as noted before, it had been obtained from an originally very virulent germ.

RESULTS OF GUINEA PIG INOCULATIONS.

The guinea pig inoculations made by one of us (Schroeder) with this attenuated germ to test its virulence were as follows: November 24, 1894, Nos. 280, 281, 282, and 283 received each an abdominal injection subcutaneously of ½ c. c. tubercle culture twenty-third generation.

On January 25, 1895, each pig again received 1 c. c. tubercle culture twenty-sixth generation. October 7, 1895, guinea pig No. 281 was found dead. A small dry, cheesy nodule at the seat of one injection was noted, but there was no other evidence of tuberculosis, and death resulted from inflammation of the bowels.

On January 3, 1896, guinea pig No. 282 was found dead from pneumonia. There was no evidence of tuberculosis. The other two pigs, Nos. 280 and 283, are alive and well at this time.

Guinea pigs Nos. 55, 56, 57, 58, and 59 were inoculated on October 2, 1895, subcutaneously with 1 c. c. tubercle culture thirty-second generation. On October 17 guinea pig No. 55 was found dead, and post-mortem revealed a general inflammatory condition of subcutaneous tissue over entire abdomen. The other animals, Nos. 56, 57, 58, and 59, are alive and well at this writing.

Again, January 25, 1895, guinea pigs Nos. 331 and 332 were inoculated with ½ c. c. tubercle culture twenty-sixth generation. On December 30, 1895, guinea pig No. 331 was found dead from pneumonia. The liver had a peculiar mottled appearance and a few yellowish patches. Though a coverglass preparation showed no tubercle bacilli, two pigs, Nos. 121 and 122, were inoculated as checks with a portion of the liver. After the lapse of six months they are alive and well and show no symptoms of disease.

On February 19, 1895, guinea pig No. 332 was also found dead, death being due to inflammation of the bowels, and there was no evidence of tuberculosis.

These inoculations made by Dr. Schroeder have confirmed the earlier experiments made by Dr. De Schweinitz, and agree with the following which have been repeated as a check on the first work. They show conclusively that from an originally virulent germ we have succeeded in obtaining an attenuated germ which, even in large doses, is apparently harmless to guinea pigs, rabbits, cattle, horses, and monkeys.

November 15, 1895, six guinea pigs were inoculated with ¾ c. c. each of an emulsion of an attenuated culture thirty-fourth generation. The pigs weighed as follows:

	Ounces.		Ounces.
No. 67	9	No. 70	11½
No. 68	11	No. 71	8½
No. 69	11	No. 81	12

November 18 No. 67 was found dead from pneumonia. The other animals exhibited the following condition on subsequent dates:

December 13, 1895.

Guinea pig.	Weight.	Remarks.
	Ounces.	
No. 68	12	Slight local swelling; no enlarged glands.
No. 69	14	Do.
No. 70	13	Do.
No. 71	10	Slight local swelling; right inguinal gland enlarged.
No. 81	14	Slight local swelling; no enlarged glands.

December 31, 1895.

Guinea pig.	Weight.	Remarks.
	Ounces.	
No. 68	14	Slightly enlarged glands.
No. 69	16	No enlarged glands.
No. 70	15	Do.
No. 71	11	Do.
No. 81	14	Slightly enlarged gland.

No tuberculosis was evident on autopsy of one of these, showing that the germ was without pathogenic effect. The animals had steadily gained in weight.

Again:

Guinea pig.	Weight.	Guinea pig.	Weight.	Guinea pig.	Weight.
	Ounces.		*Ounces.*		*Ounces.*
No. 166	15	No. 170	14	No. 174	15
No. 167	13	No. 171	11	No. 176	14
No. 168	14	No. 172	16	No. 159	10
No. 169	10	No. 173	16	No. 164	10

These animals were all inoculated on February 5, 1896, with 1½ c.c. of attenuated tuberculosis culture thirty-seventh generation. On February 13, 1896, the weights of these animals were as follows:

Guinea pig.	Weight.	Remarks.
	Ounces.	
No. 169	9	Swelling size of a pea at point of inoculation.
No. 172	15½	Do.
No. 174	15	Do.
No. 159	10	Do.
No. 170	12¼	Large swelling at point of inoculation.
No. 168	14	Slight swelling at point of inoculation.
No. 171	10	Do.
No. 173	14	Do.
No. 164	8	Slight swelling; pneumonia.
No. 166	14	Do.

On February 8 and 10, respectively, guinea pigs No. 167 and 176 were found dead from pneumonia, which they had contracted from exposure to cold. Subsequently guinea pigs Nos. 174, 172, 159, and 171 were found dead from pneumonia. On March 16, 1896, guinea pig No. 169, which had been inoculated on February 5, with the attenuated germ was found dead. There were no signs of tuberculosis, although the animal had been inoculated over a month. The pig died from pneumonia. March 10, 1896, the record of the remaining animals was as follows:

Guinea pig.	Weight.	Remarks.
	Ounces.	
No. 173	15	Slight sore at point of inoculation.
No. 169	9	No local lesion.
No. 168	16	Slight sore at point of inoculation.
No. 170	10	Apparently well.
No. 166	14	Do.

Guinea pig.	Weight.	Guinea pig.	Weight.	Guinea pig.	Weight.
March 18, 1896:	*Ounces.*	March 23, 1896:	*Ounces.*	April 7, 1896:	*Ounces.*
No. 168	16	No. 170	$10\frac{1}{2}$	No. 168	18
No. 170	10	No. 168	$16\frac{1}{2}$	No. 166	16
No. 173	14	No. 166	16	No. 170	10
No. 166	14	No. 173	$13\frac{1}{2}$	No. 173	12

There was no enlargement of the glands indicating the beginning of tuberculosis, and, except a loss of weight in pigs Nos. 170 and 173, which was suspicious, there was no evidence of tuberculosis.

These and other cases in which the guinea pigs were inoculated with the attenuated germ, and after one to two years showed no evidence of disease, have proved conclusively the attenuated character of this germ. Although this germ is so attenuated and innocuous to animals, its ability to grow in artificial media is as good and better than ever, and the tuberculin obtained from its culture is as satisfactory as that prepared from a more virulent germ.

Cows and calves have also been inoculated with this attenuated germ in doses varying from 2 to 500 c. c. at a time without the production of tuberculosis. Nodules were frequently formed at the point of inoculation, which seemed, however, to be due to the mechanical action of the germ, and did not produce any evidence of tuberculosis.

JUNE 29, 1896.

THE EFFECT OF TUBERCULIN INJECTIONS UPON THE MILK OF HEALTHY AND DISEASED COWS.

By E. A. de Schweinitz, Ph. D., M. D.

Continuing the line of experiments given in my report, Bulletin No. 7, Bureau of Animal Industry, upon the variation in the amount of fat in the milk before and after the injections of tuberculin, tests were made on different dates upon a healthy cow, No. 299, with varying doses of tuberculin, upon diseased animals Nos. 145 and 161, and also upon a set of eight different animals taken from the same milch herd—Nos. 185, 186, 187, 189, 194, 195, 222, and 234. The latter had all been condemned by the tuberculin test, and preparatory to their being killed were kept at the station for some days, thus giving an opportunity for testing their milk. There was practically no variation in the fat of the milk from the healthy cows after the tuberculin injection. This agrees with our first experiments, and also with some tests made by Dr. Law, reported in Cornell University Bulletin No. 7. Neither was there any alteration when, as is seen from the tests in March on No. 299, large doses, 30 c. c. of tuberculin, were injected. The second and third injection with tuberculin of No. 145 and 161, diseased respectively, caused no appreciable rise of temperature, but there was a decided decrease in the amount of fat.

In the series of January 16, 1895, however, the two animals that showed no rise in temperature failed to show any decrease in the milk fat. When the rise of temperature was noted in the others a marked decrease in fat was also noted.

A comparison of the decrease in fat with the extent of the disease, as revealed by autopsy and given me by Dr. Smith, except in case No. 234, a generalized one, does not apparently show any relationship. The oldest cases seemed to give the least change in fat—No. 185—while the newer cases gave the largest variation. The Tables I and II show the quantity and composition of the various samples of milk, and the Tables III and IV the temperature reactions after the tuberculin injection, for the corresponding dates.

No. 285, an animal condemned for tuberculosis about a year ago, has been kept at the station since that date. At first she was injected with small doses of tuberculin until she ceased to give a reaction and was again apparently well. The injections of tuberculin were increased in number and quantity, and on March 20, 1895, the date of the last examination of the milk, the animal received an injection of 100 c. c.

Previous to that date she had received altogether 565 c. c. of tuberculin. The last injection caused no change in the amount of fat or in the temperature.

The variation in fat should, of course, be attributed in part to the fever. But that this is not the only cause is also evident. The variation is not, judging from the few tests made, sufficient of itself to prove the presence of tuberculosis, but taken in conjunction with the rise of temperature might be considered as corroborative evidence. The tuberculin tests were made by Drs. Schroeder and Curtice, while in the milk analyses I was assisted by Mr. J. A. Emery.

In this connection are appended several charts with curves which show the average variation in temperatures of a number of different animals. These were tabulated in February, 1895, from the reports received from the different States to which tuberculin had been sent.

Chart No. 1 shows the average temperature of a number of animals, without reference to breeds. The other charts are arranged so that all animals of the same breed are placed together. The diagnoses were not in all cases proved by slaughtering the animals, but in all cases where they were killed the autopsies confirmed the diagnoses.

The weights of the animals were taken as the average weight, and the dose of tuberculin was 2 c. c. The Holstein cattle showed apparently a slightly higher reaction than the others. The temperature curve begins at the time of injection, and the first temperatures then noted are six hours after the injection with tuberculin.

The upper line in the charts shows the reaction of the diseased animals, and the lower of healthy ones. In order to make the charts smaller, an average was made of the temperatures of the different animals taken before the injection. From four to eight temperatures were taken of each animal, and the general average thus obtained with a large number of animals is given as the average normal temperature.

In the chart giving the curve for the whole number of animals there is a rise of temperature noted after twenty hours. This is due to the Holstein chart, which is included. The late reaction noted in these animals, which were from different herds, may be due to their larger size.

Many objections have been made against the use and reliability of tuberculin as a diagnostic agent, the opposition coming principally from those who are to a great extent unfamiliar with its practical use or who are only too ready to condemn a material which, through lack of skill and knowledge on their part, has perhaps given unsatisfactory results. The committee in Paris, composed of Cheveau, Leblanc, Mequin, Nocard, Strauss, Trasbot, and Weber, reported as follows upon the principal objections to the use of tuberculin: "The use of high temperatures and carbolic acid in the manufacture of tuberculin makes it impossible that the tuberculin, if properly prepared, should produce disease. It occasionally happens that tuberculin fails to give a reaction in diseased animals, but these are very exceptional cases, and occur only sometimes when the animals are very badly diseased and their condition could be easily recognized, and are not of importance. Occasionally, also, apparently healthy animals show a reaction, but when a very thorough and careful autopsy is made evidence of the disease is usually found. In a case of an apparently healthy animal, therefore, one can only say in safety that the examination had not been sufficiently close to discover the lesions. Again, in cases where there was apparently some other disease and the tuberculin injection caused a reaction a careful autopsy has shown the presence of tuberculosis, and that the reaction was due to the latter disease." This disposes of the objection that the tuberculin reaction is not characteristic.

The statement that the tuberculin injection causes the disease to spread more rapidly is not warranted by facts, and in many instances the use of tuberculin has apparently caused an improvement in the disease.

One animal, originally tuberculous, kept at the station of the Bureau of Animal Industry, has received about 3,000 c. c. tuberculin in different injections, extending over a long time. This animal is now well and fat and has entirely recovered from tuberculosis.

In 1895 the International Congress for Veterinary Medicine, at Berne, said: "Tuberculin is a most excellent diagnostic material, and can be of the utmost service in the warfare against tuberculosis." This resolution was indorsed by the French Academy of Medicine and the use of tuberculin was generally recommended. The satisfactory reports received from the different States to which this Bureau has sent tuberculin are confirmatory of the results obtained, and prove that tuberculin is the only effective means at hand to insure a rapid eradication of tuberculosis in cattle. A table showing the results of the tuberculin injection of more than 50,000 cattle will appear in the next Annual Report of the Bureau.

FEBRUARY, 1895.

TABLE I.—*Analyses of milk of cows injected with tuberculin.*

No. of animal.	Date.	Quantity.	Specific gravity.	Total solids.	Sugar.	Albuminoids.	Fat.	Ash in milk.	Total fat acid. (*a*)	Volatile fat acid. (*b*)	Acidity. (*c*)
	1894.	*c. c.*		*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*P. ct.*	*c. c.*	*c. c.*
299	Dec. 11	9,655	1.032	13.65	3.84	3.14	4.48	0.70	....	6.7	0.132
	Dec. 12	9,655	1.031	14.54	4.17	2.91	6.31	.70	....	5.0	.146
	Dec. 13	8,519	1.029	13.38	3.84	2.90	5.10	.62	....	3.9	.108
	Dec. 14	9,087	1.029	13.11	4.16	2.86	4.10	.66	....	6.6	.112
	Dec. 18	7,950	1.028	12.74	3.33	2.50	5.49	.60	....	4.7	.132
145	Dec. 11	2,000	1.018	21.61	5.00	3.21	15.34	.75	....	3.5	.100
	Dec. 12	1,785	1.025	14.68	2.94	3.13	7.73	.75	....	3.9	.136
	Dec. 13	3,407	1.026	14.95	2.38	3.49	7.16	.72	....	5.0	.130
	Dec. 14	3,407	1.027	15.03	3.12	3.51	7.21	.76	....	4.8	.134
	Dec. 18	3,407	1.027	16.26	2.77	3.75	8.18	.78	....	4.8	.114
161	Dec. 11	450	1.020	18.86	2.50	3.98	12.56	1.01	....	2.8	.072
	Dec. 12	1,025	1.027	12.99	2.63	3.05	5.52	.77	....	5.5	.072
	Dec. 13	1,703	1.026	12.77	2.77	2.99	4.09	.74	....	7.5	.102
	Dec. 14	1,703	1.026	12.92	2.94	3.06	4.91	.73	....	6.1	.110
	Dec. 18	1,703	1.026	13.73	2.63	3.18	6.35	.81	....	5.1	.090
	1895.										
299	Jan. 3	10,222	1.034	12.61	3.57	2.78	4.26	.73	....	3.3	.140
	Jan 4	9,087	1.033	12.34	3.57	2.81	3.30	.76	....	....	.150
	Jan. 8	10,222	1.032	12.45	3.57	2.96	3.87	.69	....	7.2	.168
	Jan. 9	9,087	1.034	12.58	3.84	2.13	3.86	.67	....	7.2	.156
	Jan. 10	9,654	1.033	12.95	3.84	2.51	4.06	.61	....	7.5	.154
145	Jan. 3	3,407	1.028	16.97	2.77	3.36	10.14	.86	....	1.4	.100
	Jan. 4	3,407	1.030	15.40	3.12	3.05	7.37	.76	....	1.9	.156
	Jan. 8	3,691	1.030	15.68	3.12	3.56	7.44	.75	....	1.9	.122
	Jan. 9	3,407	1.031	16.74	2.94	3.86	8.35	.73	....	4.9	.138
	Jan. 10	3,123	1.028	15.38	3.12	3.58	6.54	.70	....	5.3	.138
161	Jan. 3	1,703	1.030	17.54	2.94	3.48	9.99	.77	....	1.4	.130
	Jan. 4	1,992	1.027	13.27	3.12	2.97	5.77	.83	....	2.0	.132
	Jan. 8	2,272	1.029	14.37	2.94	3.20	6.53	.68	....	....	.124
	Jan. 9	1,988	1.031	14.63	3.33	3.35	6.52	.70	....	4.9	.130
	Jan. 10	1,987	1.031	14.57	3.33	3.28	5.91	.71	....	5.3	.124
299	Feb. 18	8,234	1.031	11.90	3.33	2.59	3.96	.68	28.39	7.4	.128
	Feb. 19	8,519	1.033	12.14	3.57	2.61	3.66	.68	30.83	7.7	.132
	Feb. 20	8,234	1.032	12.33	3.57	2.63	4.07	.68	36.60	7.0	.150
299	Mar. 4	8,519	1.031	12.29	3.57	2.72	3.87	.67	29.21	7.2	.154
	Mar. 5	8,945	1.032	11.99	3.57	2.68	3.46	.68	40.63	8.2	.156
	Mar. 6	....	1.032	12.20	3.57	2.69	3.56	.69	38.25	7.4	.160
	Mar. 8	9,087	1.032	11.98	3.57	2.61	3.25	.69	38.81	7.5	.156
285	Mar. 19	4,828	1.029	12.84	3.57	3.01	5.14	.67	27.89	5.8	.106
	Mar. 20	5,112	1.029	14.60	3.57	3.12	6.73	.67	27.21	5.2	.096
	Mar. 21	5,396	1.029	13.59	3.57	3.03	5.81	.66	22.99	5.1	.098

a Iodine absorption number.

b Number of cubic centimeters, $\frac{N}{10}$ Ba $(oH)_2$, required for 1 gram of fat.

c Number of cubic centimeters, $\frac{N}{10}$ Na oH, required for 1 cubic centimeter of milk.

TABLE II.—*Analyses of milk of cows injected with tuberculin.*

185.				189.				222.			
Date.	Quantity.	Specific gravity.	Fat.	Date.	Quantity.	Specific gravity	Fat.	Date.	Quantity.	Specific gravity.	Fat.
	c. c.				*c. c.*				*c. c.*		
Jan. 14	5,111	1.029	5.92	Jan. 14	9,655	1.030	4.28	Jan. 14	5,111	1.024	10.87
Jan. 15	4,260	1.028	5.51	Jan. 15	7,950	1.028	4.29	Jan. 15	5,111	1.024	8.20
Jan. 16	4,543	1.030	5.50	Jan. 16	7,983	1.030	3.67	Jan. 16	4,827	1.030	5.09
Jan. 17	4,543	1.030	5.71	Jan. 17	7,667	1.028	4.29	Jan. 17	3,692	1.029	6.53
Jan. 18	4,260	1.029	5.77	Jan. 18	7,667	1.027	3.68	Jan. 18	6,247	1.029	6.12

186.				194.				229.			
Jan. 14	....	....	....	Jan. 14	1,420	1.028	10.83	Jan. 14	2,556	1.023	9.03
Jan. 15	375	1.028	5.51	Jan. 15	1,704	1.025	11.06	Jan. 15	5,679	1.022	8.22
Jan. 16	500	1.022	9.04	Jan. 16	1,998	1.029	6.53	Jan. 16	5,111	1.027	4.91
Jan. 17	568	1.022	7.19	Jan. 17	2,272	1.030	6.73	Jan. 17	4,260	1.027	6.95
Jan. 18	440	1.024	6.97	Jan. 18	1,948	1.031	5.09	Jan. 18	6,816	1.029	4.28

187.				195.				234.			
Jan. 14	1,998	1.030	8.77	Jan. 14	5,111	1.024	6.01	Jan. 14	5,111	1.023	8.42
Jan. 15	3,123	1.023	12.32	Jan. 15	1,704	1.027	7.97	Jan. 15	4,543	1.023	6.36
Jan. 16	3,407	1.028	6.13	Jan. 16	2,272	1.029	4.49	Jan. 16	3,692	1.030	2.44
Jan. 17	3,408	1.030	7.54	Jan. 17	1,998	1.028	8.17	Jan. 17	3,975	1.027	7.36
Jan. 18	3,408	1.031	6.16	Jan. 18	2,556	1.030	6.52	Jan. 18	5,111	1.027	5.93

TABLE III.—*Temperature reactions of the cows used in the milk experiments.*

Date.	Time.	No. 299.	No. 161.	No. 145.
1894.				
Before injection.		°	°	°
December 11	1.00 p. m.	102.3	101.3	101.6
	3.00 p. m.	102.0	98.2	97.3
	5.00 p. m.	101.5	99.4	98.5
After injection.				
December 12 (injected 2 c. c. tuberculin at 8 p. m. December 11)	8.00 a. m.	101.0	101.0	101.4
	10.00 a. m.	100.8	101.2	101.0
	12.00 m.	101.2	100.6	102.5
	2.00 p. m.	101.0	102.0	101.2
	4.00 p. m.	101.4	101.0	101.6
1895.				
Before injection.				
January 7	1.00 p. m.	101.0	102.4	100.6
	3.00 p. m.	101.0	102.6	101.2
	5.00 p. m.	101.2	102.7	101.4
After injection.				
January 8 (injected 2 c. c. tuberculin at 8 p. m. January 7)	8.00 a. m.	101.5	101.8	105.2
	10.00 a. m.	101.0	101.7	105.0
	12.00 m.	100.4	100.8	102.6
	2.00 p. m.	101.4	101.0	101.5
	4.00 p. m.	101.3	101.5	101.1
Before injection.				
February 18	9.00 a. m.	101.6	....	....
	12.00 m.	100.6	....	....
	4.00 p. m.	101.9	....	....
	9.00 p. m.	101.2	....	....
After injection.				
February 19 (injected 2 c. c. tuberculin at 9 p. m. February 18)	8.00 a. m.	101.6	....	....
	9.00 a. m.	102.7	....	....
	10.00 a. m.	102.6	....	....
	11.00 a. m.	100.2	....	....
	12.00 m.	100.2	....	....
	1.00 p. m.	101.2	....	....
	2.00 p. m.	101.5	....	....
	3.00 p. m.	101.8	....	....
	4.00 p. m.	101.8	....	....
	5.00 p. m.	101.4	....	....
Before injection.				
March 4	8.30 a. m.	101.2	....	....
	12.00 m.	100.2	....	....
	4.30 p. m.	101.2	....	....
After injection.				
March 5 (injected 30 c. c. tuberculin March 4)	8.00 a. m.	101.5	....	....
	9.00 a. m.	102.0	....	....
	10.00 a. m.	99.2	....	....
	12.00 m.	100.2	....	....
	2.00 p. m.	101.2	....	....
	4.00 p. m.	101.5	....	....
	5.00 p. m.	101.9	....	....

TABLE IV.—*Temperature reactions of the cows used in the milk experiments.*

[First test of tuberculous cows.]

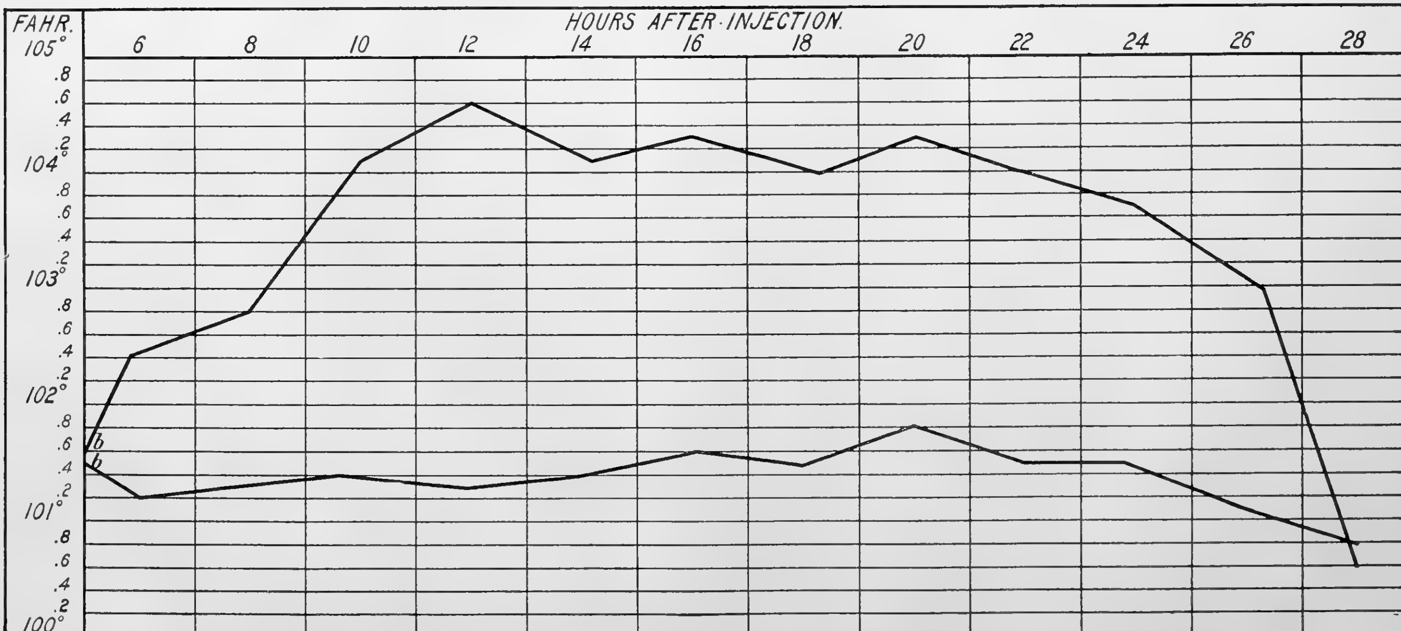
Date.	Time.	No. 185.	No. 186.	No. 187.	No. 189.	No. 194.	No. 195.	No. 222.	No. 229.	No. 234.
1894.										
Before injection.		°	°	°	°	°	°	°	°	°
December 7	9.00 p. m.	102.0	100.5	100.2	101.5	101.2	102.5	102.2	101.3	103.9
After injection.										
December 8 (injected 2½ c. c. tuberculin 11 p. m. December 7)	8.00 p. m.	102.4	101.2	101.3	102.0	102.0	105.8	102.6	105.4	103.0
	9.00 p. m.	103.1	101.4	100.3	101.8	103.8	107.0	103.6	104.8	103.6
	10.00 p. m.	105.3	103.2	102.4	104.8	104.0	107.2	104.6	103.4	103.8
	11.00 p. m.	105.6	105.6	102.1	105.8	103.8	107.2	106.4	106.2	104.8
	12.00 m.	106.6	106.0	101.5	106.6	104.3	107.4	106.2	106.9	104.5
	2.00 a. m.	106.0	105.6	99.7	106.4	106.1	106.7	106.5	106.5	105.2
	4.00 a. m.	105.4	105.1	104.7	105.6	105.2	105.3	105.0	105.4	105.7
	8.00 a. m.	103.0	106.2	104.8	105.6	102.0	104.2	105.2	104.4	104.7
	10.00 a. m.	101.0	100.5	101.0	100.5	101.0	101.1	101.0	100.4	102.6

[Second test of tuberculous cows.]

Date.	Time.	No. 185.	No. 186.	No. 187.	No. 189.	No. 194.	No. 195.	No. 222.	No. 229.	No. 234.
1895.										
Before injection.										
January 15	4.00 p. m.	102.2	102.5	101.8	102.8	102.0	101.4	101.4	101.6	100.2
	9.00 p. m.	102.0	101.8	100.8	102.5	101.8	101.8	101.0	101.0	101.4
After injection.										
January 16 (injected 2 c. c. tuberculin 11 p. m. January 15)	8.00 a. m.	101.5	102.0	99.6	102.0	102.6	105.2	104.7	103.2	105.3
	8.30 a. m.	101.8	103.2	101.0	102.0	101.8	105.2	105.2	104.0	105.4
	9.00 a. m.	102.4	103.2	101.2	103.3	102.6	105.2	105.2	104.6	105.4
	9.30 a. m.	102.2	103.5	101.2	103.2	102.8	105.3	105.4	105.4	105.8
	10.00 a. m.	102.2	103.6	101.2	103.4	102.8	105.4	105.3	105.2	106.0
	10.30 a. m.	102.2	103.0	101.6	104.2	103.0	105.2	106.0	105.5	106.0
	11.00 a. m.	102.4	103.2	101.5	104.6	104.2	105.1	106.0	105.6	106.2
	11.30 a. m.	102.6	102.9	101.2	104.2	104.4	105.0	104.6	105.4	106.4
	12.00 m.	103.0	102.8	103.2	104.2	104.4	104.8	105.3	105.6	106.3
	12.30 p. m.	103.6	102.6	103.6	104.6	104.3	104.6	105.5	104.8	106.2
	1.30 p. m.	103.5	103.0	103.9	104.8	104.6	104.5	105.5	105.2	106.0
	2.00 p. m.	103.4	102.0	104.5	104.8	104.6	104.0	104.6	104.6	105.6
	2.30 p. m.	103.6	101.6	104.6	104.4	104.2	104.0	103.4	103.5	105.2
	3.00 p. m.	102.4	100.0	105.0	100.0	102.8	103.4	103.5	102.4	104.6
	3.30 p. m.	101.9	99.5	105.0	99.6	102.4	103.0	103.2	102.5	104.2
	4.00 p. m.	101.6	99.3	104.6	99.4	102.0	103.2	103.2	102.6	104.0
	4.30 p. m.	102.8	99.6	104.5	99.4	102.0	103.0	103.2	102.6	104.2

Fig. 1.—*Chart showing average temperature of 3,930 healthy and 1,191 diseased cattle injected with tuberculin.* (*a*)

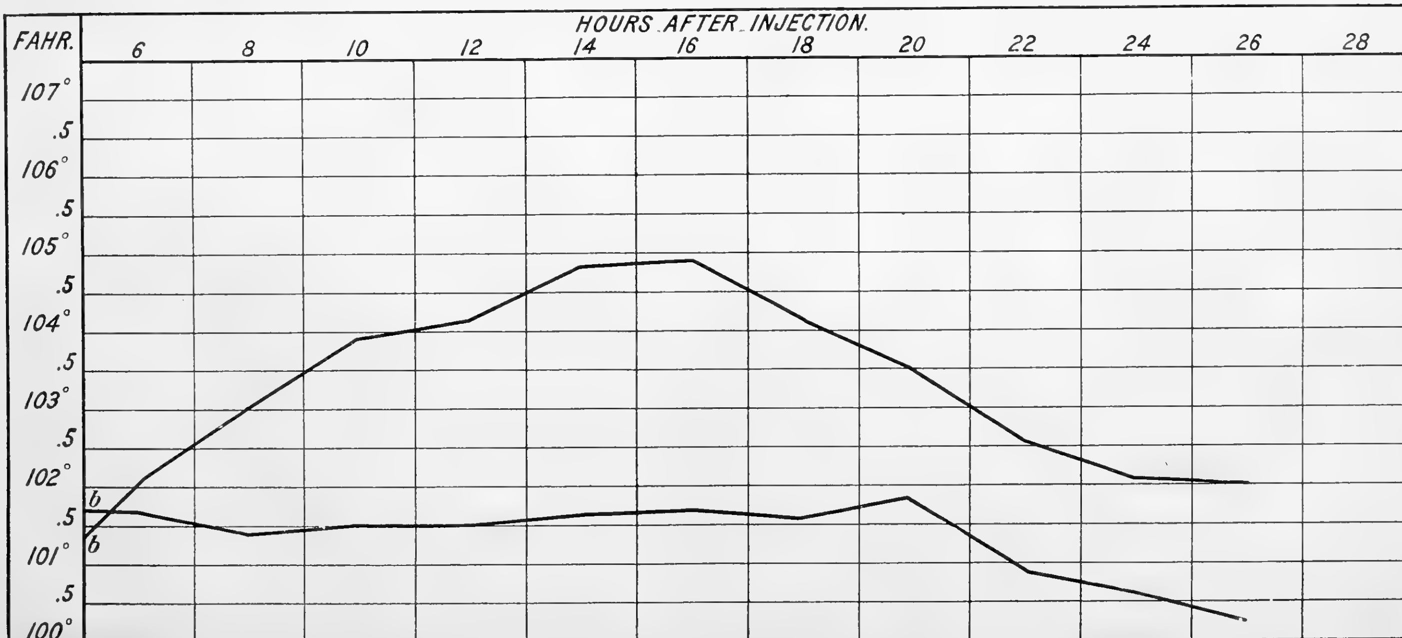
[Average normal temperature of 1,191 tuberculous cattle before injection, 101.6° F. Average normal temperature of 3,930 healthy cattle before injection, 101.5° F.]



a Tabulated in February, 1895. *b* Temperature at time of injection.

FIG. 2.—*Chart showing average temperature reaction of 62 tuberculous and 93 healthy registered Jerseys; 71 tuberculous and 303 healthy grade Jerseys; 98 tuberculous and 195 healthy Jerseys injected with tuberculin.* (*a*)

[Average normal temperature of 231 tuberculous cows before injection, 101.4° F. Average normal temperature of 591 healthy cows before injection, 101.7° F.]

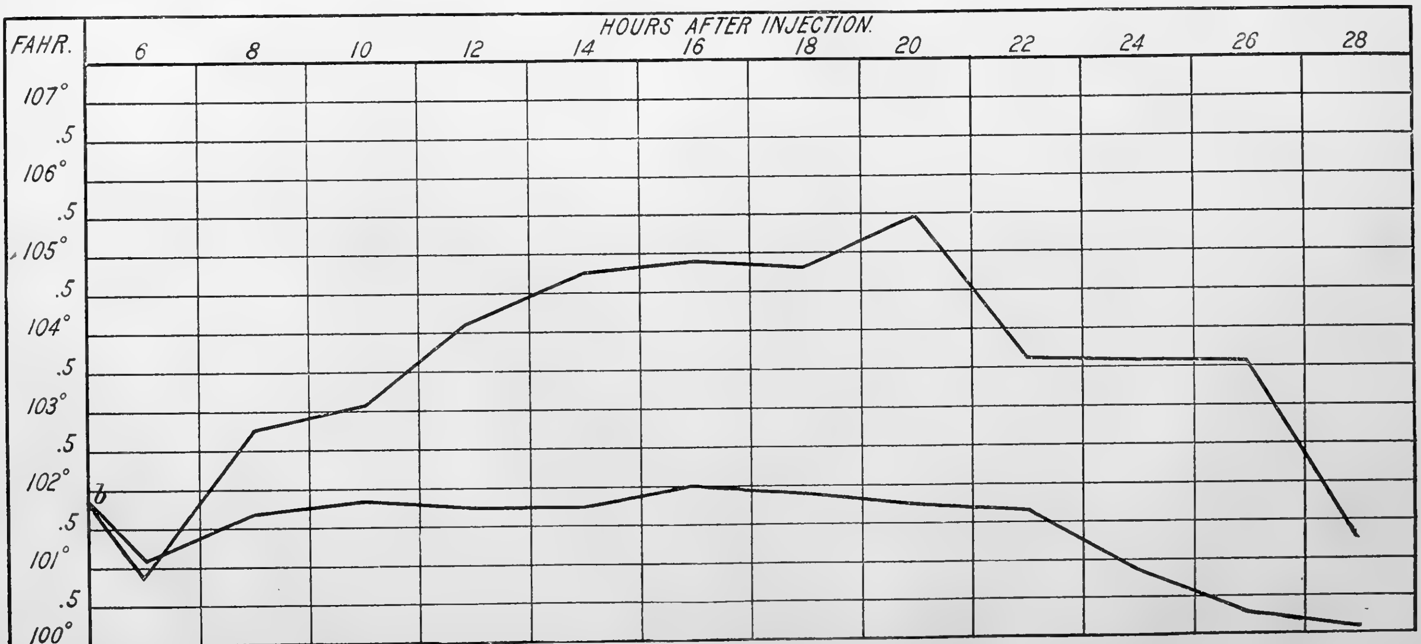


a Tabulated in February, 1895.

b Temperature at time of injection.

FIG. 3.—*Chart showing average temperature reaction of 40 tuberculous and 68 healthy grade Holsteins; 70 tuberculous and 104 healthy Holsteins injected with tuberculin.* (*a*)

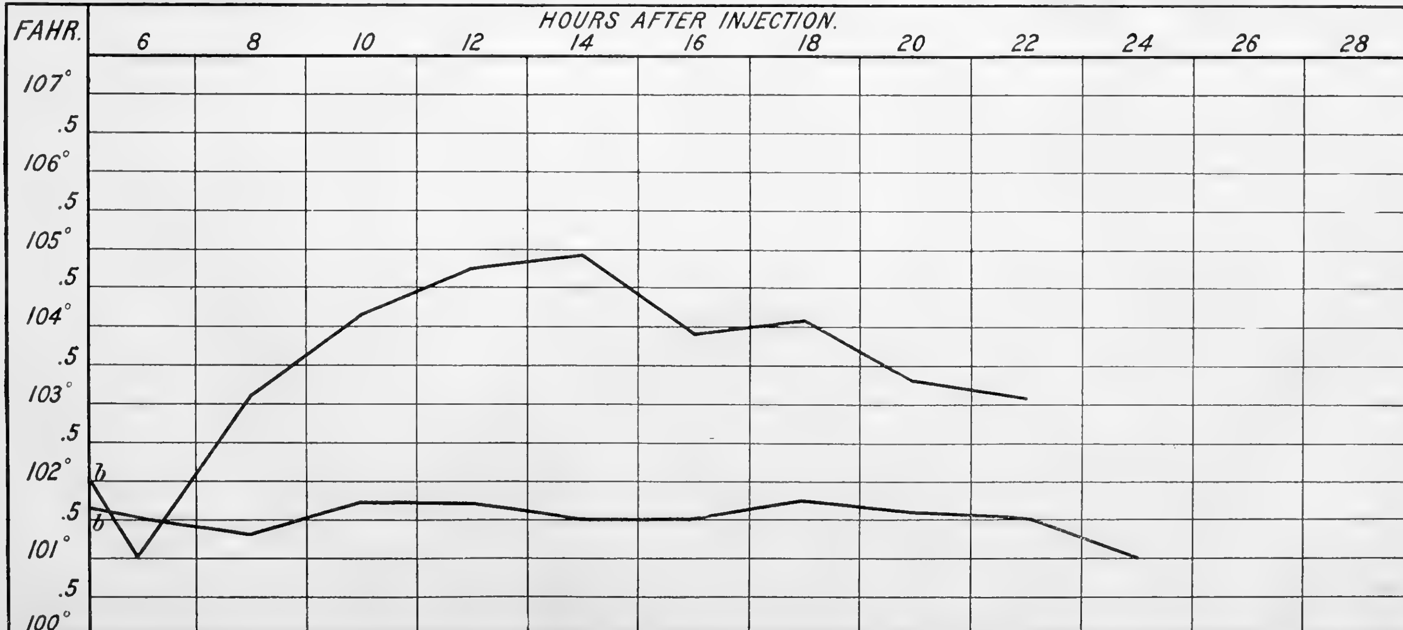
[Average normal temperature of 110 tuberculous cows before injection, 101.8° F. Average normal temperature of 172 healthy cows before injection, 101.8° F.]



a Tabulated in February, 1895. *b* Temperature at time of injection.

FIG. 4.—*Chart showing average temperature reaction of 37 tuberculous and 64 healthy Durhams; 5 tuberculous and 11 healthy grade Durhams injected with tuberculin.* (*a*)

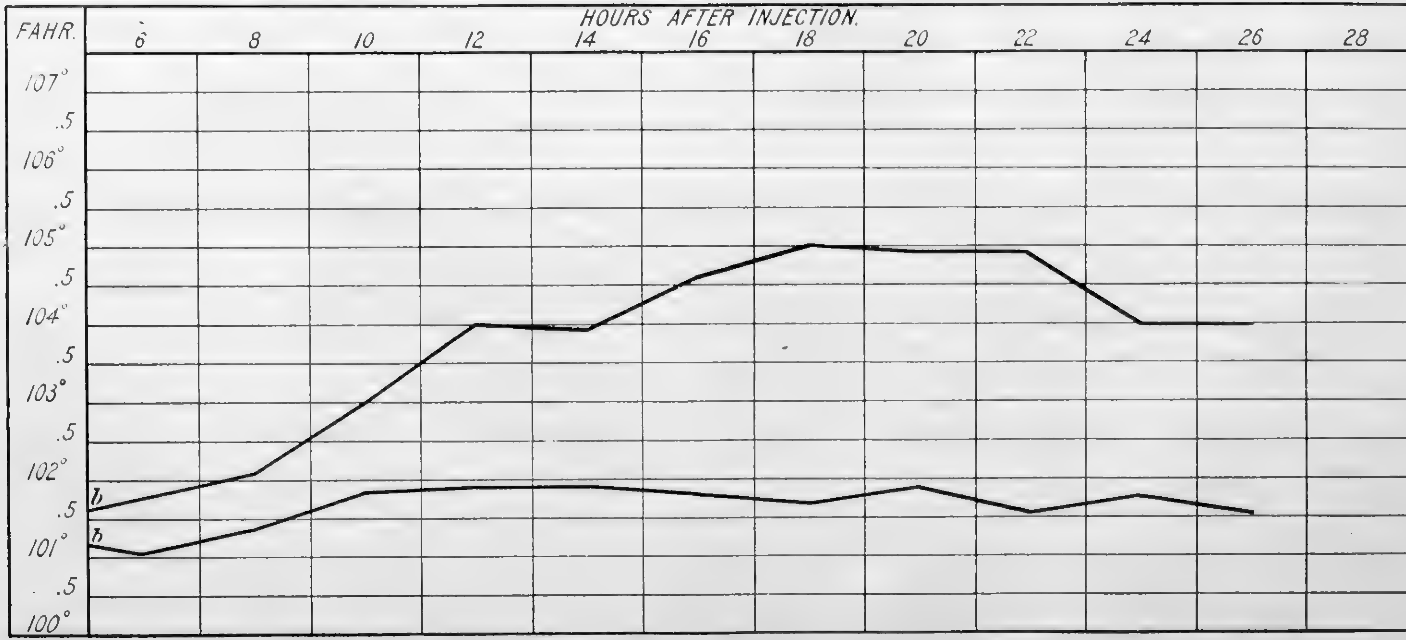
[Average normal temperature of 32 tuberculous cows before injection, 102.1° F. Average normal temperature of 75 healthy cows before injection, 101.6° F.]



a Tabulated in February, 1895. *b* Temperature at time of injection.

FIG. 5.—*Chart showing average temperature reaction of 15 tuberculous and 24 healthy Ayrshires injected with tuberculin.* (a)

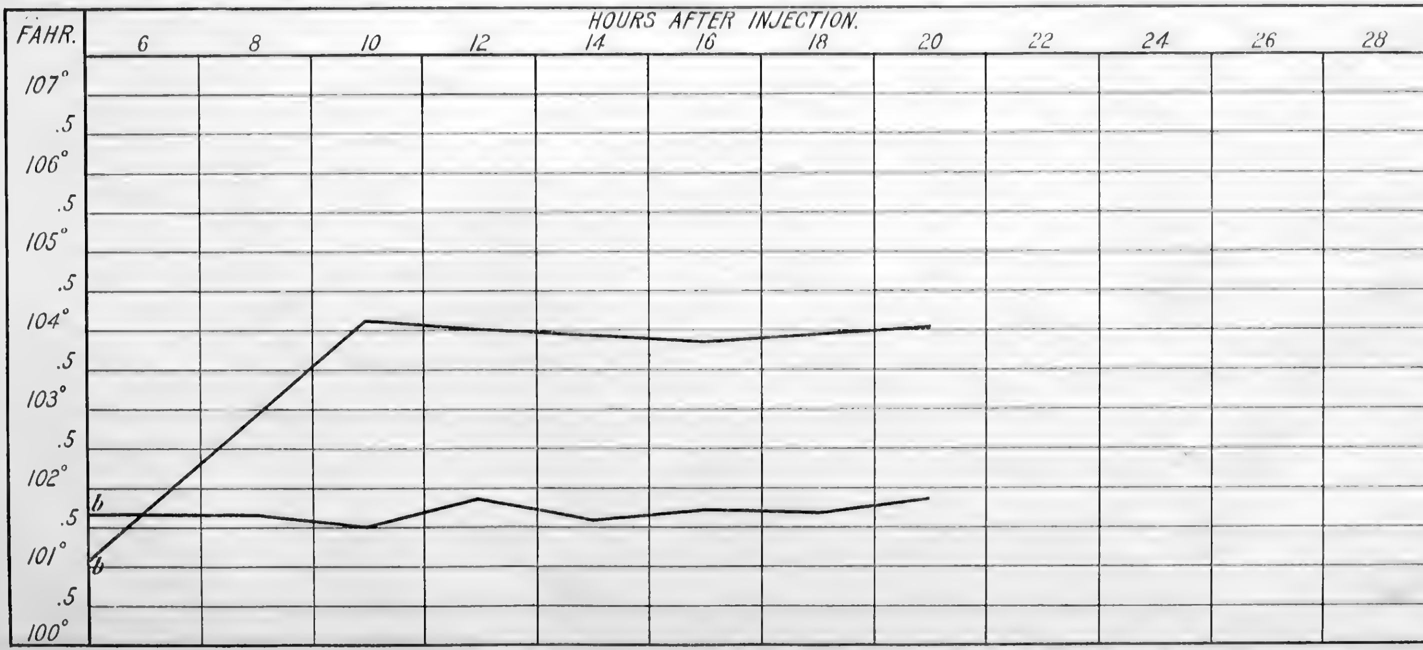
[Average normal temperature of 15 tuberculous cows before injection, 101.6° F. Average normal temperature of 24 healthy cows before injection, 101.2° F.]



a Tabulated in February, 1895.   *b* Temperature at time of injection.

Fig. 6.—*Chart showing average temperature reaction of 6 tuberculous and 108 healthy Guernseys injected with tuberculin.* (a)

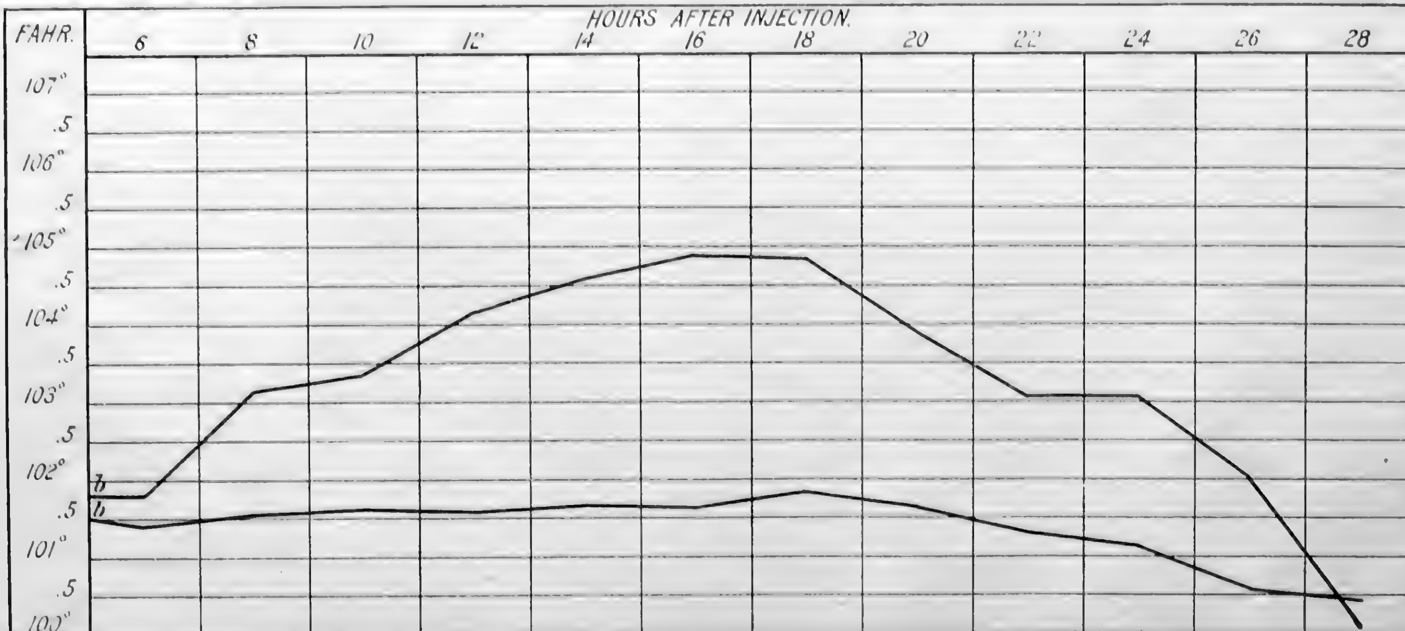
[Average normal temperature of 6 tuberculous cows before injection, 101.1° F. Average normal temperature of 108 healthy cows before injection, 101.7° F.]



a Tabulated in February, 1895. *b* Temperature at time of injection.

Fig. 7.—*Chart showing average temperature reaction of 241 tuberculous and 527 healthy grades injected with tuberculin.* (*a*)

[Average normal temperature of 241 tuberculous cows before injection 101.8° F. Average normal temperature of 527 healthy cows before injection, 101.5° F.]



a Tabulated in February, 1895. *b* Temperature at time of injection.

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