THE VACCINATION OF CATTLE AGAINST TUBERCULOSIS.*

Experiments conducted under the auspices of the Massachusetts Society for Promoting Agriculture.

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INTRODUCTORY.

The well-known principle that one attack of an infectious disease confers an immunity of shorter or longer duration is not a recent discovery. The history of medicine contains many statements which go to prove that it was known in ancient times. A knowledge of this principle probably led the Chinese into the practice of anticipating small-pox by inoculating the virus into the nose and skin and thereby producing the disease in a far less fatal form. Similar practices were in vogue several centuries ago to mitigate the ravages of animal plagues, such as rinderpest. From these dangerous practices it was but a short step to the use of so-called mitigated or attenuated viruses, — the most celebrated and successful of which is the Jenner vaccine for small-pox. This virus obtained from the cow is now generally regarded as small-pox virus, profoundly and permanently modified by its passage through the system of the cow. Though it produces in man only a local eruption it immunizes toward small-pox.

To prevent disease by protective inoculation with mitigated virus was the ruling idea in Pasteur's great work. Beginning with the use of old, spontaneously attenuated cultures of chicken-cholera for this purpose, he invented new methods for modifying and reducing virulence, and attenuated anthrax bacilli by heat, rouget in swine by passages through certain animals, and rabies by a long series of inoculations into rabbits. The rabic virus was profoundly modified by this process, and this changed virus or vaccine forms the basis

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of his successful treatment of persons threatened with rabies.

This idea of immunizing with microörganisms of reduced virulence was ever present to the minds of the earlier bacteriologists, only to be displaced for the time being by the discovery of antitoxins. For a time hope was entertained in many quarters that all infectious diseases would sooner or later be successfully combatted by the use of antitoxins, but this hope is not destined to be realized. Hence, in recent years attention has again been directed towards vaccines as preventives. While the use of attenuated living cultures is not such a safe method that it can be recommended at present for man, it is readily applicable to animals.

It is not very surprising, therefore, that the use of attenuated cultures should have suggested itself in the fight against bovine tuberculosis. Probably to most minds two difficulties presented themselves; first, that of conferring an absolute immunity against this disease, and, second, that of introducing into practice any method requiring labor and care in its application. The experiments leading up to the current method of immunizing cattle towards tuberculosis were largely tentative, and it is only within the past three or four years that the present more or less definite procedures have come into use. The preliminary work consisted: 1, in determining whether any immunity whatever was obtainable (Dixon, Trudeau, de Schweinitz); 2, whether tuberculin produces immunity and checks disease already in progress (McFadyean); 3, whether attenuated living cultures are capable of inducing any immunity (Trudeau, de Schweinitz, Pearson, von Behring, Koch and Neufeld). To settle these points various species of animals have been used, such as rabbits (Trudeau), guinea-pigs (de Schweinitz), asses and goats (Neufeld), and finally cattle. These have resulted in the use of the human type of tubercle bacilli as a vaccine for cattle.

In 1896 and 1898 the writer demonstrated the existence of a human and a bovine type of the tubercle bacillus, and showed by a series of inoculations into cattle that the human type is harmless to cattle. Starting from this point Pearson

and Gilliland[1] showed that cattle treated with human bacilli are highly resistant to bovine bacilli. Von Behring working at the same time and along the same lines, at first failed to grasp the significance of the writer's work, and it was not until 1901 that he used virulent bovine bacilli to test his vaccinated animals.* It is not necessary to go into the history in any detail, since this has been done recently by Pearson,[2] who quotes experiments in full and evidently does justice to the various investigators. So much is clear that von Behring is not the originator of the underlying principles, but he deserves great credit for his attempts to develop a practical method and make the vaccine generally accessible.

Since the earliest announcement by von Behring that a method of vaccinating cattle had been devised by him, his claims have been put to the test in different countries; in Germany by Schlegel, Eber and many others; in Austria by Hutyra; in France by Vallée; in Belgium by Degive and others. All of these experimenters demonstrated the high resistance of cattle acquired by the vaccination as determined by control inoculations with virulent bovine cultures. Later reports, however, indicate that this resistance is not sufficiently high to protect absolutely against subsequent spontaneous infection; and that the immunity acquired slowly diminishes and may disappear. The observers mentioned limited themselves to testing the cattle vaccinated according to von Behring. A more independent course was pursued by Koch[3] and his co-workers in their experiments published in 1905. These are fairly explicit as to detail, and were the first to show that a high degree of resistance may be induced in calves by two intravenous injections of any freshly isolated human bacillus. Even one injection is regarded by them as sufficient.

The procedure used by Koch and his associates is, briefly,

* In Beiträge z. Exp. Therapie, Heft 5, 1902, the tables published by von Behring show that the first attempt to isolate bovine bacilli by him was made Aug. 8, 1901, and that the culture did not grow until near the end of 1901. In 1900 he received a bovine culture from Nocard which grew luxuriantly on various media whose age and origin were unknown, and which was probably attenuated. The tabulated records do not enable the reader to determine the degree of attenuation.

as follows: The human cultures were freshly isolated from cases of tuberculosis, chiefly pulmonary in type. The bacilli were grown mainly on glycerine-bouillon. When the cultures were four to six weeks old, the masses of bacilli were removed by filtration, gently dried with filter paper, and ground with .8 per cent salt solution into a fine suspension. The injections were made directly into a jugular vein. Ten to twenty milligrams were injected the first time, and one to two months later, fifty milligrams. Forty to ninety-three days after the second injection of human bacilli, a test injection of bovine bacilli, fatal to untreated control animals, was made. Most of the animals were killed three to five months after the test inoculations. In some, tubercular foci were found. Others were free from them. These results will be referred to again after our own experiments have been described.

Hutyra[14] experimented with bovo-vaccine, with two human cultures and with one from a monkey, as vaccines. These were found to produce as great a resistance as the bovo-vaccine, a result which is corroborated in our investigations. According to Hutyra, one vaccinated animal had acquired no more immunity than a control animal possessed.

An extensive series of experiments has been recently reported by Weber and Titze,[15] who worked under the direction of the German Imperial Health Office. The original report was not accessible to me at the completion of this manuscript, and I had to content myself with abstracts. The results of these government investigations do not appear to differ from those herein described. Of importance is the statement that the immunity acquired by vaccination does not last over two years. As only one injection of fifty milligrams of human bacilli was made, there is no reason to suppose that two or three injections would not prolong the period of protection considerably. The authors call attention to the fact, to be mentioned in this paper, that vaccinated animals may offer even better opportunities for the distribution of the disease if they still have a tendency to

contract a mild type in the natural way later on. Another point of interest mentioned by these investigators refers to the prolonged survival of bacilli in the body. In one case the udder of a vaccinated cow shed human bacilli into the milk for fifteen months. This undesirable condition is not likely to be established when animals are vaccinated in early life.

In addition to the method of intravenous injection of the cultures designed to act as vaccines, several experimenters have tried the subcutaneous injection as having the advantage of greater simplicity and easier application. Baumgarten,[6] Lignières,[7] and Hutyra[8] have tried this method and favor it. Hutyra believes that it imparts as much resistance as the intravenous procedure. There is formed at the place of injection a tumor, which may discharge tubercle bacilli, besides disfiguring the animal for the time being. The method deserves careful trial to see if the lungs, which are the most frequently affected organ in the spontaneous disease, and which receive most of the vaccine by the intravenous method, are as well protected by the one as the other method.

Attempts to immunize by feeding have been made recently by Calmette and Guérin.[16] The method, for several reasons, does not appear to the writer as worthy of practical application, although the authors have presented data of theoretical interest, which have some bearing on the problem of immunity.

It would be unprofitable to review all the literature upon the subject of vaccination against tuberculosis at the present time. Some is critical, some enthusiastic, some skeptical. The time is not yet ripe for any accurate estimation of the value of this process. Those interested in the publications are referred to a recent critical summary by Eber,[4] who gives references to the printed reports of experiments designed to test von Behring's bovo-vaccine, and also to Hutyra.[5]

PLAN AND ORGANIZATION OF THE INVESTIGATION, AND METHODS EMPLOYED.

Early in the year 1906 the Massachusetts Society for Promoting Agriculture proposed to the writer an investigation of the subject of vaccination against tuberculosis. The society agreed to furnish the animals and their maintenance, while the writer was to prepare and inject the vaccines, and do all the scientific work necessary to the proper interpretation of the results. The expense of this part of the work was borne by the Laboratory of Comparative Pathology. The guiding motives of the investigations were: 1, to determine how much, if any, immunity could be induced in cattle with the use of cultures of the tubercle bacillus; 2, to test this induced immunity by cohabitation of the treated animals in herds where tuberculosis existed; and, 3, to develop out of these experiments some method of preparing a vaccine readily and cheaply. The plan finally adopted and carried out was to divide the animals into groups, and treat each group with a different vaccine, as follows:

Group A consisted of twenty-one calves vaccinated with seven different cultures of human tubercle bacilli, three animals being allotted to each culture. After two vaccinations with each culture one animal of each lot of three (in one case, two animals) was tested with a virulent bovine culture to determine the degree of immunity attained. The rest were reserved to be exposed to natural infection.

Group B consisted of three animals treated with von Behring's bovo-vaccine as a basis of comparison with Group A. Two of these were tested with the virulent bovine culture.

Group C consisted of three animals treated with large doses of virulent bovine tubercle bacilli, killed by a temperature of 60° C. All of these were tested by inoculation later.

Group D comprised three animals vaccinated with an attenuated bovine culture. One of these died, one was tested, and one reserved for exposure.

Group E consisted of five control or unvaccinated animals

used to measure the virulence of the test culture of bovine bacilli.

The calves used numbered thirty-five in all, and weighed from fifty-eight to two hundred and eighty-four pounds at the time of the first vaccination. All were grades, and most had either Jersey or Holstein blood. A commodious stable was fitted up on the estate of Mr. N. I. Bowditch at Framingham, Mass., and an attendant placed in charge of the animals. Every facility was given by Mr. Bowditch to carry on the work, and his superintendent, Mr. F. E. Barrett, assisted me in the inoculations and the autopsies, and in many other ways necessitated by the fact that the experiment was being conducted some twenty miles from the laboratory.

Some difficulty was at first experienced with the younger calves. The usual digestive troubles were encountered, and considerable care was necessary in feeding. New milk was given them for a few weeks and gradually changed for sterilized milk, to which was added a small quantity of meal. The older calves were fed skim milk, to which was added a little oil-meal jelly. In addition to this they were fed twice a day with the best rowen, and as soon as age would permit, with a small quantity of grain, consisting of equal parts of ground corn, oats and wheat. Early in the spring, alfalfa was substituted for the rowen, the skim milk stopped, and the calves, being of good size, were fed a larger quantity of grain. On the first of June all were turned out to pasture.

Owing to various unavoidable delays, the actual vaccination of calves did not begin until December, 1906. The conditions were decidedly unfavorable, and on some of the days on which the vaccinations were made the temperature was very low. Nevertheless, no untoward accidents traceable to the inclemency of the weather occurred.

Cultures used. — Seven different cultures of human tubercle bacilli and three of bovine bacilli were used for the vaccine. These cultures were isolated by the writer, and they have been described in former papers. It is therefore not necessary to give more than a few details concerning them.

These are presented in Table I. A glance at the table shows that the cultures differed widely in age, and in source. The bacilli to be injected as vaccines were obtained in all but a few cases from slanted, glycerine-agar cultures from three to six weeks old. The rest were from the membrane of bouillon cultures containing five per cent glycerine. The bacillar masses taken from the glycerine agar tubes were, as a rule, quite dry, and, therefore, no attempt was made to remove any moisture with filter paper, as is necessary where bouillon cultures are used.

TABLE I.

Designation.	Under Cultivation Since	Source. (The numbers refer to the published description.)
Human VI.....	July, 1897.	Human sputum.[8]
" X.....	Feb., 1902.	Lung tissue.[9]
" XII.....	April, 1902.	Mesenteric lymph-node, child.[9]
" XIII.....	April, 1903.	" " " " [10]
" XIV.....	March, 1903.	" " " adult.[10]
" XXIII...	May, 1906.	Lung tissue.[11]
" XXIV...	June, 1906.	" " [11]
Bovine I.....	Dec., 1894.	Cattle.[12]
" VIII.....	July, 1899.	" [8]
" IX.....	April, 1906.	" [11]

The masses of growth were removed and weighed in carefully sterilized, weighed, and covered porcelain crucibles. They were then ground in an agate mortar, and sterile normal salt solution slowly added. The final suspension was, as a rule, quite homogeneous. Occasionally a culture would tend to remain in the form of fine flocculi, although treated the same as the others. The dilution varied from one to four cubic centimeters of salt solution per centigram, according to

the dose to be injected. The suspensions were injected either on the day on which they were prepared, or, in one instance, on the day following. The mode of injection was in general that recommended by von Behring. The injection was made with syringes having glass cylinders and asbestos plungers. They were sterilized in boiling water. The left jugular vein was usually chosen as most convenient to the operator. The hair was cut from a suitable place over the vein, and the skin washed with five per cent carbolic acid. The syringe was meanwhile filled with the suspension of tubercle bacilli, and the needle removed and placed in boiling water. A second needle taken from the boiling water was first introduced into the vein upwards towards the head, while pressure was being applied below at the root of the neck to cause distension of the vein. When the blood flowed freely through the needle, the syringe was fitted to it, and the suspension slowly injected after releasing the pressure on the vein. The reason for using two needles, one to draw up the fluid and the other to insert into the vein, was to avoid introducing through the skin a needle which was covered and filled with tubercle bacilli. A local swelling might result from the deposit of bacilli in the skin and subcutis.

GROSS RESULTS OF THE VACCINATION AND OF THE TEST INOCULATION OF THE DIFFERENT GROUPS OF CALVES.

Group A. — In Tables II., III., and IV. all the data which are of importance in interpreting the vaccination and the subsequent test inoculation are gathered together. These tables show that the injection of the human cultures had no deleterious effect upon the calves. There was a steady increase in weight up to the time of the test inoculation, or the exposure of the calves to natural infection. Following the first vaccination there was in all cases a rise in temperature of variable height and duration. At first sight it would appear as if certain cultures produced a more powerful and prolonged febrile reaction than others, but a more careful examination of Table II. indicates that there is also much

individual variation among calves. The temperature record is impaired on account of the fact that the earliest vaccination in December, 1906, was preceded by a spontaneous rise of temperature among many of the calves, and that the record was not continued long enough. In several the first vaccination was followed by listlessness, loss of appetite, and cough. These, however, disappeared with the fever.

The second vaccination, which was done approximately two months later, was usually followed by a short, sharp rise of temperature, or none at all. In several instances the breathing became labored immediately after the injection. The dyspnea disappeared within twenty-four hours.

In two animals (Nos. 124, 150) vaccinated with Culture No. XIII., local swellings appeared over the site of the injection. These remained stationary for a time and then slowly subsided.

In another case (No. 32) a swelling appeared on the face about a month after the second vaccination, which later on broke and discharged a purulent fluid. Unfortunately, the inoculations into guinea-pigs were not made until three weeks after the purulent fluid had been collected and kept in a refrigerator. The guinea-pigs remained well. The stained preparations of the discharge did not reveal any tubercle bacilli.

In general, it may be said that the double intravenous injection of human cultures, both old and relatively fresh, had no injurious or retarding effect upon the twenty-one calves vaccinated.

As has already been stated, a very good means of measuring the degree of immunity in vaccinated cattle is the inoculation with bovine cultures. In order that comparable results may be obtained, such cultures must be tested upon untreated or control calves under the same or similar conditions. The culture used for this purpose was a comparatively fresh one, as it had been isolated about a year before. The inoculations in all cases were made, like those of the vaccines, into a vein. To measure the virulence of this culture five calves were used (Nos. 38, 39, 40, 41, and 42).

TABLE II.

Group A: Calves vaccinated with human tubercle bacilli.

First Vaccination.									Second Vaccination.								
No. of Calf.	Breed.	Date.	Weight in pounds.	Culture. Designation of Culture.	Culture. Age (days).	Amount Injected (Mgr.)	Febrile Reaction. Highest Temp. F.	Febrile Reaction. Length in Days.	Date.	Weight in pounds.	Change in Weight; + = Gain.	Culture. Age.	Culture. Amount Injected (Mgr.).	Febrile Reaction. Highest Temp. F.	Febrile Reaction. Length in Days.	Gain in Weight Months after First Vaccination.	Final Disposition of Animal. Remarks.
20....	Jersey Guernsey.	Dec. 8, 1906.	70	VI.	38	5	104.8*	8	Feb. 7, 1908.	131	+ 61	39	60	103	1	+ 162 (6 mos.)	June 10, exposed to natural infection.
25....	Grade Guernsey.	Feb. 7, 1907.	104	VI.	39	20	101.5	0	May 3, 1908.	158	+ 54	17	60	105	1	+ 78 (4 mos.)	June 10, exposed to natural infection.
175...	Scrub.	Dec. 8, 1906.	232	VI.	38	20	104.6	10 +	Feb. 7, 1908.	247	+ 15	39	100	104.2	3	+ 163 (5½ mos.)	Marked dyspnea for 24 hours after second vaccination. May 3, test inoculation.
28....	Mixed.	Dec. 8, 1906.	70	X.	38	10	105.6	8	Feb. 7, 1908.	121	+ 51	39	30	103	2	+ 122 (4 mos.)	May 3, test inoculation.
10....	Holstein.	Dec. 8, 1906.	205	X.	38	25	103.6	8	Feb. 7, 1908.	221	+ 16	39	50	101.8	0	+ 140 (5 mos.)	June 10, exposed to natural infection.
24....	Holstein.	Dec. 8, 1906.	76	X.	38	10	105.6*	6 +	Feb. 7, 1908.	107	+ 31	39	30	103	8	+ 106 (6 mos.)	July 1, exposed to natural infection.
23....	Grade Jersey.	Dec. 8, 1906.	58	XII.	38	10	103.8	8 +	Feb. 7, 1908.	110	+ 52	39	30	102.9	6	+ 164 (6 mos.)	June 10, exposed to natural infection.
33....	Durham.	Feb. 7, 1907.	133	XII.	39	15	103.4	2	April 19, 1908.	178	+ 45	24	60	104.8	4	+ 80 (3½ mos.)	June 10, exposed to natural infection.
146...	?	Dec. 8, 1906.	227	XII.	38	25	105*	8 +	Feb. 7, 1908.	268	+ 41	39	50	102.6	0	+ 106 (4½ mos.)	May 3, test inoculation.
124...	Scrub.	Dec. 8, 1906.	122	XIII.	38	20	106.4	11 +	Feb. 12, 1908.	152	+ 30	46	30	104.2	6	+ 89 (4½ mos.)	Dyspnea for 24 hours after second vaccination. May 3, test inoculation.
145...	Scrub.	Dec. 8, 1906.	156	XIII.	38	20	104.8	28	Feb. 12, 1908.	177	+ 21	46	35	104	20	+ 149 (6½ mos.)	Dyspnea for several hours after second vaccination. June 28, test inoculation.
150...	?	Dec. 8, 1906.	188	XIII.	38	20	104	4	Feb. 12, 1908.	226	+ 38	46	50	102.4	0	+ 160 (6 mos.)	June 10, exposed to natural infection.
31....	Grade Jersey.	Feb. 12, 1907.	205	XIV.	27	20	104.8	16 +	April 19, 1908.	250	+ 45	34	40	105.4	2	+ 104 (4 mos.)	October 17, exposed to natural infection.
32....	Grade Jersey.	Feb. 12, 1907.	149	XIV.	27	15	105	16 +	April 19, 1908.	210	+ 61	34	40	104.4	3	+ 92 (4 mos.)	October 17, exposed to natural infection.
37....	Holstein Jersey.	Feb. 12, 1907.	219	XIV.	27	22	105.2	6	April 19, 1908.	288	+ 69	34	40	105	5	+ 146 (4 mos.)	June 28, test inoculation.
137...	Holstein Jersey.	Dec. 12, 1906.	140	XXIII.	21	20	104.4	30 +	Feb. 12, 1907.	158	+ 18	43	35	103.4	6	+ 103 (5 mos.)	May 3, test inoculation.
172...	Grade Guernsey.	Dec. 12, 1906.	138	XXIII.	21	12.5	105	4	Feb. 12, 1907.	179	+ 41	43	35	102.6	0	+ 179 (6 mos.)	June 10, exposed to natural infection.
185...	Grade Durham.	Dec. 12, 1906.	218	XXIII.	21	25	104.8*	7 +	Feb. 12, 1907.	246	+ 28	43	50	104	5	+ 152 (5½ mos.)	June 10, exposed to natural infection.
14....	Jersey Holstein.	Dec. 12, 1906.	139	XXIV.	42	10	105*	6	Feb. 12, 1907.	191	+ 52	46	30	104	3	+ 141 (4½ mos.)	Chills after second vaccination. May 3, test inoculation.
132...	Scrub.	Dec. 12, 1906.	203	XXIV.	42	20	103.2	4	Feb. 12, 1907.	250	+ 47	46	50	104.2	4	+ 191 (6 mos.)	June 10, exposed to natural infection.
173...	Grade Durham.	Dec. 12, 1906.	187	XXIV.	42	20	104.2*	3	Feb. 12. 1907.	237	+ 50	46	50	104.2	2	+ 187 (6 mos.)	June 10, exposed to natural infection.
Group B: Calves vaccinated with von Behring's bovo-vaccine.																	
30....	Grade Guernsey.	Feb. 7, 1907.	240	Bovine vaccine.	Feb. 18 (limit).	2 units.	103.4	1	April 19, 1907.	284	+ 44	May 5 (limit).	5 units.	103	2	+ 111 (4 mos.)	June 28, test inoculation.
34....	Grade Durham.	Feb. 7, 1907.	154	Bovine vaccine.	Feb. 18 (limit).	1.5 units.	105.8	3	April 19, 1907.	251	+ 97	May 5 (limit).	5 units.	106	2	+ 140 (4 mos.)	October 17, exposure to natural infection.
36....	Grade Holstein.	Feb. 7, 1907.	195	Bovine vaccine.	Feb. 18 (limit).	1.5 units.	105.4	2	April 19, 1907.	269	+ 74	May 5 (limit).	5 units.	104.4	2	+ 134 (4 mos.)	June 28, test inoculation.
Group C: Calves vaccinated with heated bovine tubercle bacilli.																	
11....	Scrub.	Dec. 8, 1907.	211	Bovine VIII.	Heated 60 minutes.	130 mgr.	105.6	9	Feb. 7, 1907.	246	+ 35	Heated 45 minutes.	222 mgr.	102	0	+ 151 (4½ mos.)	May 3, test inoculation.
12....	Grade Jersey.	Dec. 8, 1907.	284	Bovine IX.	Heated 60 minutes.	100 mgr.	103.4*	4	Feb. 7, 1907.	336	+ 52	Heated 45 minutes.	400 mgr.	101.9	0	+ 130 (4½ mos.)	May 3, test inoculation.
35....	Grade Guernsey.	Feb. 7, 1907.	161	Bovine VIII.	Heated 45 minutes.	78 mgr.	102.9	0	April 19, 1907.	215	+ 54	Heated 40 minutes.	200 mgr.	104	2	+ 91 (4 mos.)	June 28, test inoculation.
Group D: Calves vaccinated with attenuated living bovine bacilli.																	
13....	Jersey Holstein.	Dec. 12, 1906.	136	Bovine I.	42 days.	2.5	104.6	12 +	.....	.....	.....	.....	.....	.....	.....	+ 134 (4½ mos.)	May 3, test inoculation.
118...	Jersey Holstein.	Dec. 12, 1906.	134	Bovine I.	42 days.	5	104.6	10 +	.....	.....	.....	.....	.....	.....	.....	+ 136 (6 mos.)	October 17, exposed to natural infection.
184...	Scrub.	Dec. 12, 1906.	200	Bovine I.	42 days.	10	106.2	40 +	.....	.....	.....	.....	.....	.....	.....	.....	January 17, died.

*A distinct elevation of temperature a few days before the vaccination makes the value of these figures somewhat uncertain.

They are recorded in Table IV. as controls. The dose of bacilli injected varied from 2.5 to ten milligrams. It will be noted that the smaller dose was more quickly fatal in summer than in the spring. This may perhaps be due to other causes than the season — such as natural resistance of the animal, change of feed, etc. In general, the doses given were fatal in fifteen to thirty days. It is probable that much smaller doses would have proved fatal, though after a somewhat longer period. The dose of ten milligrams is thus equivalent to at least four times the dose necessary to kill, and it may be ten times that dose.

Of Group A, eight animals were selected to undergo this severe test. Nos. 14, 28, 124, 137, 146, and 175 were inoculated on May 3, 1907, about two and five-sixths months after the second vaccination, with ten milligrams of the bovine culture, and Nos. 37 and 145 were inoculated with five milligrams of the same culture June 28, *i.e.*, about two and one-third months after the second vaccination. Of the first lot, No. 124 died in ten days; No. 28 in thirty-three days, and No. 137 in forty-three days. No. 14 was killed after three months, Nos. 146 and 175 after six months. Compared with the controls, No. 124 died sooner, Nos. 28 and 137 somewhat later. The remaining three resisted, and slight traces of disease were found at the autopsy. The cause of the sudden death of No. 124 is not clear. The writer did not see the autopsy. The microscopic examination of the lung tissue indicated that the disease in the lungs was under way, but not severe enough to prove fatal.

Of the two inoculated June 28 with the smaller dose of five milligrams, No. 145 died in forty-two days, and No. 37 resisted and was killed after four and one-half months. In the latter only very slight lesions were found.

The reasons for the irregular outcome of the test are several. There were seven human cultures used, of widely different ages and from various types of disease. The dosage also varied considerably and there was probably more or less difference in the natural resistance of the animals. One fact seems to stand out fairly well in Tables II. and III.,

and that is the evident relation between size of the vaccinal dose and the acquired immunity. Thus, No. 175 vaccinated with Human VI., the oldest and most attenuated human culture used, resisted, but it had received twenty and one hundred milligrams. Cultures No. XXIII. and No. XXIV. were fresh cultures, but No. 137 received only twenty and thirty-five, and No. 14, ten and thirty milligrams respectively. Another fact is the evident failure of Culture No. XIII. to immunize as well as the other cultures.

Group B. — This group of three was vaccinated with bovo-vaccine (von Behring). All the necessary data are given in Tables II. and III. In order to make these tests as like the others as possible, the animals were carefully chosen and all procedures were carried out with the utmost care. Owing to the fact that for the first vaccine only a five-unit dose was at my disposal, it was divided among the three, so that two received one and one-half units each, the third two units instead of the prescribed one unit. At the second vaccination, each received five units as prescribed. Two of the three were selected for the test inoculation, No. 36 receiving ten, No. 30, five milligrams. No. 36 died in thirty-nine days, and No. 30 resisted to be killed after four and one-half months. Slight traces of tuberculosis were found in the lungs, udder, and meninges of the brain. Without going into further details, I may simply state that a careful study of the tables does not show any superiority of the bovo-vaccine over my Cultures Nos. VI., XII., XIV., and XXIV. A higher dosage of Cultures No. X. and XXIII. would probably have brought their effect up to the level of the others.

Group C. — These were vaccinated with bovine bacilli killed by heat at 60° C. The object of this experiment was to learn to what degree the injection of heated cultures used to such an extent in the treatment of human tuberculosis* might increase the resistance of calves. Inasmuch as such a slight impact as that due to the injection of living human

* The ground bacilli (Tuberculin R.) or the bacillar emulsions in the market are, as a rule, heated at 60° C. before they are injected into human patients to avoid the possibility of introducing living germs.

cultures produces a marked increase in resistance, it was anticipated that the injection of five to ten times the number of virulent bovine bacilli killed at 60° C. might produce a similar result. Three animals, Nos. 11, 12, and 35, were used for this experiment. Two were treated with Bovine VIII., one with Bovine IX. By this arrangement it was hoped to bring out any differences in vaccinating power which might exist in cultures recently and more remotely isolated from the cow, Bovine VIII. having been over seven years, Bovine IX. about seven months under cultivation.

The bacilli were removed from glycerine-agar cultures, suspended in normal salt solution and heated in closed, submerged test-tubes in a water bath at 60° C. for from forty to sixty minutes. As pointed out by the writer some years ago, the tubercle bacillus is killed at 60° C. in twenty minutes, and in the present instance, even very dense suspensions of the bacilli were made harmless as shown by the absence of any untoward effect on the inoculated animals. The doses given were from five to ten times those of the living human bacilli. The intervals between the first and second injection of heated cultures, and between the latter and the test inoculation was the same as for the other groups. Tables II. and III. contain all the data relating to the injections and the results of the final test with virulent bovine bacilli.

The temperature of No. 11 rose on the second day after the first vaccination, reaching a maximum of 105.6° F. on the third day, and gradually subsiding to normal, within nine days. There was no rise after the second injection. No. 12 behaved like No. 11, but the temperature did not go higher than 103.6° F. and fell quite rapidly. No. 35 showed no reaction whatever after the first injection, and a very slight rise after the second; also, immediately after the second injection labored breathing which soon subsided. The animal refused to eat and appeared dull and listless for twenty-four hours. No such symptoms were noticed in Nos. 11 and 12.

TABLE III.

Treated (or vaccinated) calves subjected to test inoculation with virulent bovine bacilli (Bovine IX.).

Groups A, B, C, and D.

No. of Calf.	Mode of Vaccination.	Date of Test Inoculation.	Culture Used.		Weight at or Near Time of Test.	Weight at Death, or when Killed.	Result.	Remarks.
			Dose (mgr.).	Age (days).				
175...	Human VI.	May 3, 1907.	10	17	382	399	Killed Nov. 8 (6 months).	In lungs numerous subpleural collapsed lobules having translucent gelatinous appearance. Similar condition in one caudal lobe, forming mass the size of fist. Slight pleural and peritoneal tuberculosis. Some grayish dots or striæ in medulla of kidneys.
28....	Human X.	May 3, 1907.	10	17	192	166	Died June 5 (33 days).	Anterior half of both lungs solidified. Miliary tuberculosis. Thoracic lymph nodes, large, necrotic. Diffuse fatty degeneration of liver.
146...	Human XII.	May 3, 1907.	10	17	359	359	Killed Nov. 5.	After inoculation, two swellings about size of hen's egg appeared at site of injection. Both completely softened at autopsy. Lymph nodes of neck not affected. In lungs scattering collapsed lobules and some of a yellowish color. In several thoracic glands very small, completely calcified nodules.
124. .	Human XIII.	May 3, 1907.	10	17	211	.....	Died May 13 (10 days).	(Histological examination only.) Exudative, desquamative, and tubercular lesions — the latter not conspicuous. Fibrin in alveoli among groups of desquamated cells and polynuclear leucocytes. Tubercle bacilli few. Central necrosis and fatty degeneration of liver lobules.
145...	Human XIII.	June 28, 1907.	5	18	305	275	Died Aug. 9 (42 days).	(Histological examination only.) Sections show more or less exudation into alveoli associated with many tubercles. These contain large numbers of giant cells. Fatty degeneration of liver.

37....	Human XIV.	June 28, 1907.	5	18	360	361	Killed Nov. 1.	Several calcareous nodules in left retropharyngeal lymph node. Papillomata of pharynx. Two very small foci in kidneys. Several subpleural collapsed lobules in lungs. A sessile, flat tubercular mass, size of silver half dollar, on pleura.
137...	Human XXIII.	May 3, 1907.	10	17	243	.....	Died June 15 (43 days).	Body found in state of advanced decomposition. Histological examination of lungs shows immense number of spore-bearing anaërobes. Structural details effaced. Tubercle bacilli detected. Probably advanced tuberculosis.
14....	Human XXIV.	May 3, 1907.	10	17	280	.....	Killed Aug. 15 (104 days).	Breathing slightly labored and temperature somewhat elevated when killed. Scattering collapsed lobules of grayish red color in one cephalic lobe of lungs, and a few in other lobes, found to contain tubercles on microscopic examination. This reveals also very minute foci in kidneys and thoracic lymph nodes.
30....	Bovo-vaccine.	June 28, 1907.	5	18	350	311	Killed Nov. 4.	Scattered widely over all lobes are collapsed lobules due to tubercular process as shown by histological examination. Several small foci in kidneys. Beginning tuberculosis of udder and meninges, and retropharyngeal glands.
36....	Bovo-vaccine.	June 28, 1907.	10	18	323	.....	Died Aug. 6 (39 days).	Tubercular pneumonia of anterior half of lungs.
11....	Bovine bac. (heated).	May 3, 1907.	10	17	362	.....	Died May 22 (19 days).	Tubercular pneumonia of anterior half of lungs. Great hyperplasia of thoracic lymph nodes. Uniform fatty degeneration of liver. Entire lobule involved. Beginning tubercles seen in sections of spleen, and in some lymph nodes.
12....	Bovine bac. (heated).	May 3, 1907.	10	17	414	333	Killed May 22, in advanced stage of tuberculosis.	Lesions same as in preceding case.
35....	Bovine bac. (heated).	June 28, 1907.	5	18	252	.....	Killed July 19, in failing condition.	Lesions as in No. 11. Not quite so far advanced.

The test inoculation of virulent bovine bacilli was followed in all three cases by elevation of temperature, which continued high until one (No. 11) died, and the others were killed. These latter were at the time they were killed in an advanced stage of the disease. The breathing was very labored and the animals were killed to avoid suffering. A comparison of the three cases as regards temperature, symptoms, rapidity of the disease, and the condition of the organs, especially the lungs, after death, with the animals which had not received any preliminary treatment reveals very little, if any, difference worth noting. In short, the injection of large masses of virulent bovine tubercle bacilli killed at the lowest effective temperature failed under the conditions of the experiment to produce any appreciable resistance in these animals. If any resistance can be brought about in this way, more delicate methods will be required to reveal it.

Group D. — Of considerable interest are the cases which were vaccinated with a bovine culture whose virulence had become attenuated by prolonged cultivation. This culture was isolated by the writer in 1894. It was, therefore, just twelve years under artificial cultivation; the culture having been renewed by transfer of a particle of the growth to a fresh tube approximately every month during this period.

Three animals only were inoculated with this culture (Nos. 13, 118, and 184). They received respectively two and one-half, five, and ten milligrams. In spite of the prolonged cultivation of this bacillus, it had maintained enough virulence to kill the calf receiving the largest dose in thirty-six days. After the inoculation this animal (No. 184) maintained a temperature ranging between 103° and 104° F. After seven days the temperature was not taken until on the twenty-third day, when it was 106.2° F. and it remained high until death. Symptoms of disease were first noticed on the nineteenth day, when the animal appeared dull and listless. Several days later the breathing was noticeable and became quite labored until death.

TABLE IV.

Controls inoculated with virulent bovine bacilli (Bovine IX.).

Group E.

No. of Calf.	Breed.	Weight.	Culture Used.		Date of Inoculation.	Result.	Remarks.
			Dose (mgr.).	Age (days).			
38....	Grade Jersey.	200	10	16	April 11, 1907.	Died in 18 to 19 days.	Extensive disseminated tuberculosis of the lungs, associated with pneumonic condition of the cephalic (or anterior) half of both lungs. Fatty degeneration of the liver. Many tubercle bacilli in the tubercles of the lungs.
41....	Grade Jersey.	210	2.5	16	April 11, 1907.	Died in 27 to 28 days.	Same as No. 38.
39....	Grade Holstein.	209	10	17	May 3, 1907.	Died in 20 days.	Same as No. 38.
40...	Grade Jersey.	178	5	18	June 28, 1907.	Died in 15 days.	Same as No. 38.
42....	Grade Jersey.	155	2.5	18	June 28, 1907.	Died in 15 days.	Same as No. 38.

The autopsy showed extensive formation of tubercles, one to two millimeters in diameter in the lungs. The anterior half was deeply congested and not normally collapsed. Other organs were not notably affected. On microscopic examination many bacilli were found in the more or less diffusely necrotic tubercles, and fibrin was fairly abundant in the form of solid moulds filling up a small proportion of the alveoli. There was thus no question as to the immediate or remote cause of death.

The calf (No. 13) which received the smallest dose of the attenuated bovine culture had only a moderately high temperature after inoculation, which subsided within a week. There was noticed, however, a dry, husky cough for nearly three weeks. There was a steady gain in weight. (On Dec. 29, 1907, the weight was one hundred and forty-seven pounds. On April 30, 1907, two hundred and seventy pounds.) On May third this calf with a number of others received a test inoculation of ten milligrams of a relatively fresh virulent bovine culture (Bovine IX.) into a jugular vein. A control calf (No. 39) weighing two hundred and nine and one-half pounds which received an equivalent dose at the same time died in twenty days. Beginning on the second day the vaccinated calf had a high temperature, reaching a maximum of 105.4° F. in the evening of the fifth day, and gradually subsiding until a normal level was reached on the eighteenth day. Taken at intervals thereafter the temperature did not register above normal. The weight, however, showed the effect of the virulent culture. On April thirtieth, three days before the test inoculation, it was two hundred and seventy pounds. On May fifteenth, during the febrile period following the inoculation, it was two hundred and fifty. On June eighth, it was again two hundred and seventy. Between this latter date and October seventeenth, it, together with the other calves which survived the test inoculation, gained but a few pounds, whereas the gain among the calves (Nos. 31, 32, and 34) simply vaccinated with human cultures, averaged thirty-six pounds during the same period. From October thirty-first to

November tenth, the temperature taken twice daily was normal throughout. On November tenth a dose of tuberculin, prepared by the writer from human bacilli, was injected. On November eleventh the temperature rose within thirteen hours of the time of injection to 105° F. During this febrile reaction, the calf was killed and carefully examined. The only lesions seen with the naked eye were as follows:

In the left half of the udder a firm mass about seven by four centimeters in size. On section it is made up of firm, circumscribed areas (lobules), each of which has in its center an irregular cavity (milk duct) containing an opaque creamy fluid. No necrosis as yet recognizable. Surrounding the focus and separating it from the still normal udder tissue is a layer of edematous hyperemic tissue about six millimeters thick, due, presumably, to the tuberculin reaction. The microscopic examination of the purulent fluid in the ducts of the affected focus was negative as regards tubercle bacilli, but two guinea-pigs inoculated with it, became tuberculous. Cultures made from them were positive and contained a tubercle bacillus, which from its luxuriant growth and its virulence was most like the test culture injected last The only other macroscopic lesions were a limited number of small, grayish nodules in the medullary and cortical portions of both kidneys.

The animal (No. 118) receiving the next largest dose of the attenuated bovine culture was one of the light-weight animals. Immediately following the vaccination the temperature rose to 106.6° F. in the same evening, and gradually fell to normal within a week. The attendant reported rather quick, unnatural and somewhat labored breathing for several months after inoculation. At the same time the temperature was normal. In August, 1907, about eight months after inoculation, the animal did not look very well, and the winter's coat had not been fully shed. The weight, however, had steadily increased. In October this animal was in good condition; it had gained in weight nearly as much as the other vaccinated calves. Thus, during the period from June

twenty-ninth to October seventeenth, Nos. 31, 32, and 34 had made an average gain of thirty-six pounds; No. 118 had gained twenty-eight pounds. It was thought best to save it for a natural exposure.

This experiment is of interest in showing, first, that bovine tubercle bacilli may be attenuated by prolonged cultivation, for the culture in question (Bovine I.) had a virulence of one-fifth to one-tenth that of the recent culture (Bovine IX.); in the second place it shows that the attenuated bacillus in a single small dose accomplishes the same as two injections of human bacilli in four to many times the quantity; in the third place, the attenuated culture still possesses, after twelve years of cultivation, a virulence not only far above the human cultures, but evidently of a different quality.

A similar experiment with an attenuated bovine bacillus was described by Koch and his co-workers.[3] The origin of the culture used by them is not given. It is far less virulent than the one used by me, for a calf receiving twenty-five milligrams was only temporarily affected thereby. Hutyra[5] repeatedly injected virulent bovine tubercle bacilli into a calf weighing two hundred and twenty pounds. He began with two milligrams, and increased the dose to ten, fifty, and, finally, one hundred milligrams in the course of seventeen months. When killed two years and seven and one-half months after the first injection, the animal weighed one thousand thirty pounds. It was found healthy.

The fifteen remaining calves of which thirteen had been treated with human cultures and one each with bovo-vaccine and bovine cultures were distributed among different herds. These animals will be kept under observation for several years and their condition will form the subject of another report.

A BRIEF DESCRIPTION OF THE INOCULATION DISEASE IN CALVES.

Inasmuch as the different publications dealing with bovine vaccination contain very meager, if any, statements on the

pathological condition of the calves used in the investigations, it seemed desirable to present very briefly the results of observations during life, and of histological investigations made of the tissues after death.

This phase of the investigations is important, because the inoculation disease produced by the intravenous injection of bovine tubercle bacilli is quite different from the spontaneous disease and cannot, therefore, be compared directly with it, for the tubercle bacilli injected into a jugular vein first reach all parts of the lungs. Here they are partly screened out and the rest are carried into different parts of the body. The tubercle bacilli, therefore, begin their attack in many points at the same time, whereas in the spontaneous disease only a few gain entrance at a time and start from a single point, or at most a few points.

The general character of the lesions found in animals which succumbed to the test inoculation, or which were killed in a failing condition, varied with the duration of the disease. In those animals which died at nearly the same period after the test inoculation, the lesions were practically in the same stage, no matter how the calves had been vaccinated beforehand. I refrain, therefore, from giving autopsy notes of individual cases, except to point out a few special features.

Those animals which succumbed to the test inoculation within fifteen to thirty days showed signs of labored breathing some days before death. At the autopsy the lungs, as might be anticipated from the mode of inoculation, presented the severest lesions. The anterior half of both lungs (including both cephalic, ventral, and an adjacent third or fourth of both main or caudal lobes) was bright or bluish red, firm to the touch and sank in water. This pneumonic condition was uniform as to distribution in all cases. The remainder of the caudal lobe was usually distended, moist, and edematous. Throughout the entire lung tissue could be seen, on section, gray tubercles, one to two millimeters in diameter, not very numerous. Besides these, a careful examination revealed countless gray points in the lung

tissue, often seen distinctly only with a hand lens. The larger tubercles, relatively few in number, were probably due to clumps of tubercle bacilli forming emboli in the blood vessels, the minute ones to bacilli lodging in the capillaries. The air tubes contained a frothy fluid.

The bronchial and posterior mediastinal lymph nodes which drain the lung tissue were, as a rule, very large,—perhaps ten or more times the normal size. On section there were numerous large grayish yellow areas in them.

Of the remaining organs, the liver was the only one clearly diseased. It was usually pale, with a yellowish tint. On microscopic examination extensive fatty degeneration was found. The lesion radiated from the central vein of the lobule, and extended close to the periphery. In several cases the fatty change was associated with areas of cell necrosis, at or near the central vein. Tubercles were rarely found.

In sections of the lung the tissue was studded with tubercles often only one or two alveoli apart. These tubercles were about two to four alveoli in diameter, either isolated or confluent, forming groups. They were made up of epithelioid cells, whose nuclei were already undergoing degeneration. Among them were polynuclear leucocytes. Giant cells were absent. In the alveoli surrounding the tubercles there were masses of coarse granules, fibrin, either as a compact mould or as a network, polynuclear leucocytes, and desquamated cells. In the pneumonic area, the capillary network was greatly distended with red cells, and the endothelium was proliferating, as shown by occasional mitoses.

The lesions in the associated lymph nodes were like those in the lungs, and need no special description.

Though the other organs were but little changed in these early cases, yet tubercles were occasionally seen in kidneys, spleen, and lymph nodes generally. The bacilli were very numerous, and well stained in the tubercles of this group of cases.

In one case (No. 124) death unexpectedly occurred as

early as ten days after the test inoculation. I did not see this case, but tissues placed in fixing fluid for me showed that the disease of the lungs was of the same character as described above, with the exception that the tubercles were not yet so sharply outlined from the surrounding tissue. There was less indication of degeneration, and polynuclear leucocytes were more abundant. Tubercle bacilli were still rather scarce as compared with the former group, indicating that active multiplication had not yet set in. The liver in this case showed extensive fatty degeneration and areas of intralobular cell necrosis.

In the case which succumbed in thirty-three days (No. 28), the central necrosis of the tubercles was now established. Giant cells, though in very small numbers, were appearing. The liver tissue shows both fatty degeneration and cell necrosis. In this group of cases, tubercle bacilli may be very abundant in the tubercles, but they are, as a rule, very thin, feebly stained rods, and not readily seen. Only a small number are normally plump and well stained.

Linking this case with the last group to be described is No. 145, which died in forty-two days after the test inoculation. In this case, the tissue immunity had gone much further. The pulmonary lesions are well established, and there are numerous tubercles in the parenchyma, but there is little necrosis. On the other hand, giant cells are present in large numbers. Some tubercular foci are made up solely of them. The interference with respiration due to the many tubercles throughout the lungs was evident in the condition of the liver. There was slight general fatty degeneration, and the cells of the central zone of many lobules were being compressed and destroyed by the passive congestion of the organ. Tubercle bacilli were found in very small numbers.

The next and last group to be considered includes those animals which survived the test inoculation apparently uninjured, and were killed three and one-half to six months thereafter. Among these were Nos. 13, 14, 30, 37, 146, and 175. Some of these, as will be seen from the table, received but half as large a test dose as the others. In all these cases

tubercular lesions were found at the autopsy. In some they were very slight, not recognizable to the unaided eye; in others they were more conspicuous. In all they were atypical, not like the lesions usually encountered after natural exposure. This may be attributed to the increased resistance following vaccination.

The lungs, being the organs first reached by the bacilli after intravenous injection, were slightly affected in all cases. In No. 13 the affection was so slight that it was detected only in sections with the microscope. The lesion here found is, in fact, the underlying characteristic lesion of this group. It is a hyperplasia of the peribronchial lymphoid tissue. This was present but slightly in No. 13. There were no distinctly epithelioid cell foci, merely the increase in lymphoid cells. In all other cases the process had gone further than this. The hyperplasia was more extensive, and occasionally it was associated with epithelioid cell areas within the lymphoid tissue. It surrounded and sheathed the intra- and inter-lobular bronchi, up to one millimeter in diameter, more rarely the larger ones provided with cartilage. This hyperplasia caused a deformation of the air tube, and the growth breaking through the epithelium invaded the lumen of the tube, thus partly or wholly occluding it. In these tubules could be seen cylindrical plugs of epithelioid cell masses, often attached to and continuous with the subepithelial tissue, and permeated loosely with polynuclear leucocytes. As a result of this proliferation into the lumen of the bronchioles, the lobule or lobules supplied by them collapsed. The walls became approximated and passively congested. Some of these lobules, through aspiration, developed intra-alveolar tubercles, usually with large giant cells. In others there resulted a broncho-pneumonic focus with exudation of fibrin and leucocytes.

This process, as studied in sections, and showing different stages in the separate cases, gave rise to the scarce or more numerous small flesh-red patches on the surface of the lungs representing collapsed lobules. Inasmuch as broncho-pneumonic foci of this character are very rarely seen in

spontaneous pulmonary tuberculosis of cattle, except in association with larger tubercular foci, where the new cell masses rapidly become necrotic, the process above described must be regarded as very slow. In five of this second group the lesions originated four and one-half to six months before, yet they had the appearance of recent lesions, for in most of the cases there was no sign of necrosis or death of tissue. The only other explanation possible is that some of the injected virulent bacilli were not destroyed, and remained inactive; that the tissue did not respond by proliferation until shortly before the animals were killed. Though I am inclined to the first hypothesis, yet neither can be proved without a careful study of a series of cases, treated exactly alike, and killed, one at a time, at regular intervals. This point is of great importance since the latency and subsequent multiplication of tubercle bacilli in the tissues would, if proved, destroy much of the value of the vaccination. It would, in fact, mean that the immunity was only temporary.

The other organs affected in all cases of the second group are the kidneys. Small circular or elongated grayish foci, one to several millimeters in diameter, were found in the cortex or the medulla. There were also microscopic foci present. In no case were they numerous; usually there were only a small number in a kidney. These foci were made up of masses of cells resembling lymphoid cells, where the foci were very small. The larger contained also areas of epithelioid and more rarely giant cells. There was no necrosis, nor any indication of rapid or even slow growth. Though the tissues were relatively fresh, *i.e.*, fixed in Zenker's fluid from one-half to one hour after the animal had been killed no mitoses were seen. The larger (one to three millimeters) foci did a certain amount of damage in destroying the secreting tubules which lay within their territory. The glomeruli remained intact even after the convoluted tubules were seen only in remnants.

The spleen was not enlarged. The Malpighian follicles, quite distinct and with germinal centers well defined, did not reveal any characteristic epithelioid cell formation.

The liver, in several cases, showed a few small collections of lymphoid cells. Beyond this the organ was normal.

In one case (No. 30) there were minute grayish bodies along the vessels at the base of the brain. In sections, my suspicions were confirmed by finding in the meninges in the adventitia of the small vessels groups of epithelioid and typical giant cells containing numerous, well-stained tubercle bacilli. It is of interest to note here that Hutyra[5] lost two cases as a result of a tubercular meningitis of the base of the brain. They both had been vaccinated, and the disease developed several months after the test inoculation.

In two cases the udder was the seat of tubercular lesions. In No. 13 there was a firm indurated mass within one quarter, about seven by four centimeters in diameter, already described. In sections the microscope showed small necrotic areas in the secreting structures, as well as small groups of epithelioid and giant cells. The acini within the indurated mass are imbedded in an edematous stroma in which there is much hyperplasia of lymphoid tissue, and invasion by polynuclear leucocytes. The small ducts are partly filled with cell masses closely resembling those found in the tubercular broncho-pneumonia of these cases. In No. 30 the udder lesions were microscopic and not recognized at the autopsy. The walls of the ducts leading from the acini are converted into broad bands of lymphoid tissue. Many giant cells are scattered through the interstitial tissue between the ultimate secreting structures. The characteristic lesions are the lymphoid hyperplasia and the giant cells. Necrosis was not observed. After prolonged search two well-stained bacilli were found.

The lymph nodes of this last group of cases were not enlarged, except the pubic or udder gland of No. 13, in which the tubercular focus was found. In two cases completely calcified nodules were found. These, to be referred to again, I am inclined to attribute to a spontaneous infection, probably before the vaccination had been begun. Whatever other lesions were found consisted of very small groups of epithelioid cells, more rarely with giant cells

situated near the peripheral lymph sinus, or more deeply imbedded in the lymphoid tissue. The germinal centers of the follicles were conspicuous, and rather sharply demarcated from the peripheral zone of the follicles.

In No. 146, a surviving vaccinated case, two large caseous tumors developed at the place of injection of the virulent test culture, probably owing to some escape of the culture fluid into the subcutaneous tissue from the vein. The point of interest in this case was the absence of any infection of the cervical lymph nodes below these tumors. This I interpret as a result of the immunity, for ordinarily such lymph nodes become diseased.

In this last group of cases, surviving the test inoculation, tubercle bacilli were very scarce and found only after considerable search. Inoculations into guinea-pigs were not made, except in one case, because it was assumed on the basis of the work of others that all lesions however small, which were not completely calcified, contained living tubercle bacilli. Pearson and Gilliland[13] found even the calcified nodules infectious to guinea-pigs. In their cases, however, the animals were continuously exposed to infection.

Thus far no mention has been made of the temperature reactions of the different groups of cases to the test inoculation. Since the animals were kept on the pasture during June and after July tenth, the temperature records of the later cases are incomplete, but enough can be gleaned from the figures at hand to enable us to differentiate the groups fairly well.

In all of the controls (Group E) the test inoculation was followed by a gradually rising, somewhat fluctuating fever, with the highest temperature of 105.5° to 107.2° F. near death. The high level of fever generally was established at the end of the first week.

The animals of Group C, vaccinated with heated bovine bacilli, reacted like Group E. The temperature did not rise at once to a maximum, but more gradually.

All the other vaccinated animals, Group A, B, and D, had a different temperature curve. There was a prompt elevation

following the injection, reaching 105° F. This we may regard as a tuberculin reaction, the tuberculin being in the bacilli injected. It fell in one or two days to 103° to 104.5° F., where it remained ten to eighteen days, and then subsided to normal or slightly above. In those animals which survived three and one-half to six months and then were killed, it remained low with an occasional rise of one or more days in No. 146. In those vaccinated animals which died in thirty to forty-five days the temperature again rose shortly after the first decline, and remained high till death.

It would seem that the vaccinated animals overcame the injected tubercle bacilli enough to cause a temporary or permanent subsidence of the fever. In some cases the injury inflicted upon the lung tissue in this struggle was so great that the process was lighted up again, and the animal with lungs greatly injured steadily failed. This is well illustrated in No. 137. This calf's temperature fell to normal on the tenth day, and remained low. Death was, therefore, unexpected, and probably due to pneumonia, which was added to the existing tubercular lesions.

GENERAL SUMMARY AND INTERPRETATION OF RESULTS.

A comparison of our results with those of Koch and his coworkers indicates on the surface that he had produced a greater resistance in his vaccinated animals. Passing by the fact that he used larger vaccine doses in his second injection than in most of our cases, we may say that it is impossible to make accurate comparisons because of possible differences in the virulent control cultures used. The table which he gives of the untreated or control calves inoculated with his culture indicates a highly virulent one, but the weights of the calves used are not given, nor the total age of the culture. This was at least fourteen months old when used first, and nearly two years old when used on the last lot of calves. How much older than this it may have been is not stated. The culture used by me was twelve and fourteen months old when injected.

If we examine the tables published by Hutyra,[5] we learn that his test culture was much less virulent than mine. Thus, of four animals which received twenty milligrams two died in twenty-nine and forty-one days respectively, and two lived two and one-half and three and one-half months respectively, and were then killed. With this culture he was also able to inject five milligrams without producing any serious disease, and, by repeating the injection, to give finally a dose of one hundred milligrams, whereas in my series two and one-half milligrams of the virulent culture was fatal within thirty days. It is probably not going wide of the mark in stating that Hutyra's virulent test culture corresponded to my attenuated Bovine I. Hutyra[14] furthermore states that von Behring's virulent bovine culture kills cattle of two hundred and forty to four hundred and twenty pounds in weight, intravenous, in doses of twenty-five milligrams, in three to six weeks. One milligram may kill young animals in five to six weeks. This culture is probably of the same virulence as the one used by me. It will thus be seen that comparisons of experiments made with different test cultures cannot well be made unless these cultures have themselves been tested side by side. Yet the crux of the whole matter lies with these test cultures.

Our results warrant the statement which has been made quite uniformly by the Commissions and other public bodies who have investigated von Behring's methods, and whose reports have appeared since this work was begun, that typical human tubercle bacilli act like his bovo-vaccine in producing a high degree of resistance in cattle. The actual degree may vary somewhat with the cultures used, and may be above or below that produced by bovo-vaccine. In animals which have survived the test inoculation four or more months, there are usually some lesions, either visible to the eye or microscopic. The broncho-pneumonic lesions, however slight, lead to mechanical and other injuries which favor any residual tubercle bacilli, and the process is likely to extend. The same is true of the lesions in the udder, where the secreting structures become injured by compression,

and the ducts clogged and ulcerated. In both situations pus bacteria may enter to extend the process. In spontaneous infection the udder is only reached, if at all, in the later stages of the disease through the blood and therefore need not be considered, but the lungs are usually attacked, and any lesions starting like the ones described in the peribronchial tissue are likely to remain and extend.

The resistance of vaccinated cattle to the spontaneous disease is reported by nearly all experimenters as not absolute. The acquired immunity slowly declines with time. There is no need for our taking any position on this phase of the question at present, but there are two points worth noting. Infection occurring in the natural way has its origin in very few bacilli, possibly only one. It may be that the same animal is infected several times. But the number of bacilli which enter the system is very small as compared with the millions or billions of bacilli introduced by the test inoculation. If these can be overcome until only a very few are left, as in those cases which were killed by us after three to six months, it would seem as if absolute immunity might be attainable. But there are several factors which are militating against this desirable condition: 1, The high virulence of the bacillus as it comes directly from the diseased tissues in contrast with the cultivated bacillus which probably loses much of its virulence in the early months of its artificial life, may largely account for failures when the test inoculations are so promising; 2, the opportunities offered the tubercle bacillus by pathological conditions of the lungs, such as lobular pneumonias due to exposure, aspiration of foreign bodies, etc., may overcome the tissue immunity in some cases; 3, the mechanical injuries due to a very little disease in the lungs favor further extension of the disease process. These conditions must be reckoned with and, pending the collection of more evidence concerning spontaneous infection of vaccinated cattle, we should not place our hopes too high.

The dangers arising from the presence of partly immune cattle in a herd are probably greater than under ordinary

conditions. These animals may discharge bacilli from some local focus without losing in condition. They may, of course, be detected with tuberculin, provided the reaction has not been modified by immunity and still serves as a fairly safe guide in such cases, a point not yet fully cleared up. Vaccination, once introduced, must therefore be applied to all animals alike, and especially to the young stock, if there is any suspicion of infection in the herd, for eventually the tubercle bacillus of the resistant animals may be expected to rise in virulence and become more dangerous to the unprotected stock, in accordance with the laws governing infectious agents.

These and other considerations have induced most experimenters in recent years to utter the warning that the methods of vaccination in use have not yet yielded such perfect results as to enable us to dispense with the other preventive measures suggested by Bang, for example. In fact it is urged that general preventive measures cannot be dispensed with at the present stage of inquiry. Vaccination may be found greatly to assist in general prophylaxis, and the two used together may finally rid a herd of infection without the resort to drastic measures.

The possible curative influence of the injection of human tubercle bacilli upon an existing early tuberculosis is of considerable interest, and has been referred to by other writers from time to time. The most extensive experiments to probe this phase of the subject were made by Pearson and Gilliland.[13] They took groups of calves which reacted to tuberculin, and repeatedly injected either human bacilli alone or these and tuberculin at intervals. The results of the autopsies upon the treated and untreated animals living with them show in the former group marked tendency towards repair, and indications of healed lesions. The evidence presented by these experiments goes to show that it is possible to arrest tuberculosis, but not to rid the animal of living bacilli. The important query which arises is — Can two, or possibly three, injections of bacilli arrest the disease in its early stages? For it would be unprofitable to give as many

injections as Pearson and Gilliland have made. They have shown that it might be done, even if not in every case, and it remains to be determined whether it may be accomplished with the current methods of vaccination. In our series tuberculin was not used at the outset to determine any prior infection in the experimental calves. The only evidence encountered by me as pointing towards a curative influence of the vaccination was the existence of very small, completely calcified tubercles one to two millimeters in diameter in the retro-pharyngeal lymph nodes of No. 37, and in the dorsal mediastinal nodes of No. 146. These I look upon as healed lesions of an early infection, and it is reasonable to assume that the vaccination and subsequent inoculation are responsible for the repair.

In the further development of the method, and for the purpose of determining its precise value and its proper place in the protection of cattle against tuberculosis, several lines of trial or investigations might be profitably carried out.

1. In the case of valuable animals, the degree of immunity at present attainable by two intravenous injections of human tubercle bacilli might be fortified and reënforced three or more months after the second vaccination, by an injection of attenuated bovine tubercle bacilli, such as those of Bovine I. The precise dose could be readily determined by using as a basis the foregoing experiments. These experiments show that five milligrams were readily borne by an untreated animal, and half that dose by itself produced a high degree of resistance.

2. In the case of ordinary stock, the method of vaccination might be used to eliminate animals of a low degree of natural resistance by the use of a single injection of an attenuated bovine culture. Animals might be treated with a given dose, and those that succumbed would eliminate themselves. Those that fell away in flesh should be destroyed. In our own experiments, among Nos. 13, 118, and 184, I believe that 184 was less resistant than the rest, although the proof is not absolute. Such a process of elimination may eventually lead to a stock naturally more resistant to

tuberculosis. There is every reason why cattle should be bred for resistance to disease as well as for the production of milk, butter, and beef.

The objection to the use of attenuated bovine cultures, which may be urged, is the possible danger of reintroducing the disease in the vaccine. This I do not believe could occur with a culture such as Bovine I. Though it might kill in large intravenous doses, I doubt that it could produce any spontaneous infection, inasmuch as two of our animals disposed of millions of bacilli. Only by carefully executed artificial transfers through a series of calves could any return to its original virulence be made possible.* However, I do not recommend the use of attenuated bovine cultures in unskilled hands, or in herds without proper supervision until the culture has been watched for several years, and more tests have been made with it.

CONCLUSIONS.

1. Vaccination of calves with the human type of the tubercle bacillus is harmless. Cases in which injuries are said to have resulted from it may have been due to other concomitant affections, among which pneumonia is probably the most common. Persons trying vaccination should first assure themselves that the culture they intend to use belongs to the human and not to the bovine type of the bacillus.

2. Vaccination with the human type of bacillus leads to a relatively high resistance to fatal doses of the bovine bacillus.

3. Vaccination with a carefully tested, attenuated bovine

* As is well known, the bovine bacillus grows very slowly and feebly in cultures when first isolated from cases of disease. The test culture used in these investigations (Bovine IX.) though still highly virulent, grew very richly as a result of one year's artificial life. This culture was injected into Calf No. 13, May 3d. On November 11 tissue from the tubercular udder of this case was inoculated into guinea-pigs. On December 24, cultures were made from one of them. These cultures grew very richly, although the bacilli had been in the body of the calf over six months, and in the body of the guinea-pig for more than a month. In other words, there was no indication even in this relatively young culture of a return to the condition in which it is found in cattle after a sojourn of six months in the body of a calf. How much less chance for a culture, twelve years under cultivation, to return to its former habits by passages through one calf, or even several calves in succession!

bacillus may be as efficacious even in a single injection, as the double vaccination with human bacilli. Such vaccination may be less dangerous to man than when human bacilli are used.

4. The immunity conferred by vaccination, as hitherto practised, does not appear to be satisfactory as regards degree or duration. More evidence is needed with regard to these points. The herds of large public institutions are well adapted to decide these questions if vaccination is thoroughly applied, and the animals supervised by properly trained men.

5. Insufficient immunity following vaccination may prove dangerous in giving rise to mild cases, after ordinary exposure in infected herds, which tend to discharge tubercle bacilli from small foci in the lungs.

6. The immunity acquired by two vaccinations with human bacilli should be fortified by a subsequent injection of attenuated bovine bacilli.

7. Investigations should be made looking toward the selection, by the injection of attenuated bovine bacilli, of races or breeds of cattle which possess naturally a high degree of resistance to tuberculosis. The capacity of different breeds to acquire a high degree of immunity should also be investigated.

8. The survival of human and bovine bacilli in the lungs and udders of calves vaccinated intravenously with them should be more definitely determined.

9. Vaccines may be easily and cheaply prepared in the form of suspensions in fluids ready for injection. The length of time during which suspensions maintain their highest efficiency remains to be determined.

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