STUDIES ON THE PATHOGENESIS OF THE AVIAN TUBERCLE BACTERIA.

Thesis for the degree of
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by

Lawrence A. Mosher.

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STUDIES ON THE PATHOGENESIS OF THE AVIAN TUBERCLE BACTERIA.

In December, 1913, Sir John M'Fadyean (1) of the Royal Veterinary College, London, England, completed a series of experiments relating to the vaccination of cattle against tuberculosis.

Previous to his experiments Von Behring (2) showed that a degree of immunity could be produced in calves against tuberculosis by the intravenous injections of attenuated cultures of human organisms, that had been grown for a number of years on artificial media. Unfortunately, this period of immunity was found to be one of short duration, and in order to successfully combat tuberculosis by this method it would be necessary to make yearly vaccinations. Later this procedure was found to be more or less dangerous in as much as the organisms were in some cases continually being given off by some of the animals, more especially in the milk.

Review of Literature.

The work carried on by M'Fadyean was to deter-

mine the efficiency of intravenous injections of avian organisms, for immunization of calves against tuberculosis. In addition to the injection of the avian organism, injections of the human bacterium were made for comparative purposes.

In his work he assumed the avian organism to be nonpathogenic to the bovine species as far as producing any serious disease was concerned. But unfortunately, from a scientific point of view, he did not conduct any control experiments to show whether or not a tubercular condition could be produced in calves by this method of vaccination.

He first grew the tubercular organism on five percent glycernated broth. The surface growth was removed and weighed, then injected intravenously into calves, in doses ranging from 1050 milligrams. The calves were at least three months old before injections were made. After a period of a few months, the calves were injected intravenously with 2-4 mg. immunity of tubercle bacteria of bovine origin to see if an immunity

had been produced. It was found that 3-4 mg. of the bovine culture was too severe a dose causing death unless the animal was killed in a morbid condition when at the point of death. The remainder of the calves, with the exception of two, were injected subcutaneously with varying doses as it was thought that the immunity could be more accurately measured by this means. These two were injected 9 months after the first inoculation with 2 mg. of tubercle bacteria.

In a large percent of the calves the subcutaneous inoculation had no effect other than producing a local lesion at the point of injection. At times it was noticed after the injection of the avian vaccine, that some of the animals showed an elevation of temperature and often a cough developed which usually lasted for only a few days. After a period of about a year the animals were killed and in most cases slight lesions were found in some of the organs. Whether this condition was due to avian vaccine or to the injection of the bovine culture is a question worthy of the utmost consideration.

Record of M'Fadyean's Experiments.

Series I.

In this series eleven calves were used which were injected with the bovine tubercle bacteria on the 9th of Feb., 1912. Nine of the calves were injected with either human or avian organisms as the table will show, while the remainder were used as controls.

Series I.

Tabulations of Injections.

No.	Date of 1st.vac.	Nature of bact.	Dose in mg.	Date of 2nd vac.
8	10/8/11	Human	10	____
12	10/8/11	Avian	10	2/11/11
15	10/8/11	Avian	10	2/11/11
16	10/8/11	Avian	10	2/11/11
20	10/8/11	Avian	10	____
56	Controls (unvaccinated)			
6	10/8/11	Human	10	____
11	10/8/11	Avian	10	2/11/11
19	10/8/11	Avian	10	2/11/11
25	10/8/11	Avian	20	____
59	Control	(unvaccinated)		

Tabulations of Injections, (continued).

No.	Nature of bact.	Dose in mg.	Date of test inj.	Dose in mg.	Date of death.
8	———	—	9/2/12	3	15/3/12
12	Avian	20	9/2/12	3	26/2/12
15	Avian	30	9/2/12	3	26/2/12
16	Avian	30	9/2/12	4	27/2/12
20	Avian	40	9/2/12	4	26/2/12
56			9/2/12	2	25/2/12
6	———	—	9/2/12	5	22/7/12
11	Avian	20	9/2/12	5	8/12/13
19	Avian	40	9/2/12	10	23/7/12
25	———	—	9/2/12	5	23/7/12
59			9/2/12	5	22/7/12

*Calves 8, 12, 15, 16, 20 and 56 were injected intravenously.
Calves 6, 11, 19, 25, 59 were injected suboutaneously.

Series I.

Tabulated results.

No.	Liver.	Lung.	Spleen.	Kidney.	Pleura.
8	0	+	0	+	0
12	+	+	0	0	0
15	+	+	0	0	0
16	0	+	0	0	+ (over lung)
20	+	+	0	0	+
56	0	+ (epicardium involved)	0	0	+
6	+	0	+	0	+
11	0	+	+	0	0
19	+	+	0	0	0
25	+	0	+	0	0
59	+	+	+	0	+

Series I, Tabulated results, (continued).

No.	Peritoneum.	Lymph nodes.	Condition.
8	0	Bronchial Mediastinal	Poor
12	0	Mediastinal	Poor
15	0	Mediastinal	Poor
16	0	Mediastinal	Poor
20	0	Mediastinal	Poor
58	0	Mediastinal	Poor
6	0	Mediastinal	Good
11	0	Mediastinal Prescap. Pharyngeal	Good
19	0	Bronchial Prescap. Portal Mediastinal	Good
25	+	Pharyngeal Bronchial Mediastinal Hepatic	Good
59	+ Parietal	Tracheal Pectoral	Poor

Series II.

This series represents fifteen calves, five of which had been vaccinated with the human type of tubercle bacteria, (one once, and four twice), while seven had received two injections of the avian organism. Of the remainder, two were used as controls, which along with the vaccinated animals received test inoculations with the bovine tuberoular bacterium on the 18th of April, 1913. Calf (44 a) was also unvaccinated and was used as a control on the 2nd test inoculation, having been injected intravenously with bovine tuberoular bacterium. Test inoculation 44 a was made intravenously, the remainder were subcutaneous.

Tabulations of Injections.

No.	Date of 1st vac.	Nature of bac.	Dose in mg.	Date of 2nd vac.	Nature of bac.
10	10/18/11	Human	10	---	---
37	25/11/11	Human	10	15/1/12	Human
39	25/11/11	Human	15	15/1/12	Human
42	25/11/11	Human	20	15/1/12	Human
44	25/11/11	Human	20	15/1/12	Human
17	10/18/11	Avian	10	2/11/11	Avian
18	10/18/11	Avian	10	2/11/11	Avian
22	10/18/11	Avian	10	2/11/11	Avian
23	10/18/11	Avian	5	2/11/11	Avian
47	25/11/11	Avian	20	19/1/12	Avian
50	25/11/11	Avian	20	19/1/12	Avian
54	25/11/11	Avian	2L	19/1/12	Avian
57	Controls (unvaccinated)				
58	Controls (unvaccinated)				
44a	Controls (unvaccinated)				

Tabulations of Injections, (continued).

No.	Dose in mg.	Date of test inj.	Dose in mg.	Death.
10	____	18/4/12	5	3/12/12
37	20	18/4/12	5	13/12/12
39	25	18/4/12	10	24/7/12
42	30	18/4/12	10	25/7/12
44	30	18/4/12	5	19/6/13
17	30	18/4/12	5	3/12/12
18	30	18/4/12	5	16/12/12
22	40	18/4/12	5	—
23	50	18/4/12	10	24/7/12
47	30	18/4/12	5	3/6/13
50	40	18/4/12	5	16/12/12
54	50	18/4/12	10	25/7/12
57		18/4/12	5	2/12/12
58		18/4/12	10	13/6/12
44a		2/1/13	1	25/1/12

Series II.

Tabulations of results.

No.	Lung.	Liver.	Spleen.	Kidney.	Perito-neum.	Pleura.
10.	0	0	0	0	0	0
27.	0	0	0	0	0	0
39.	0	+	0	0	0	0
42.	0	0	0	0	0	0
44.	+	+	+	0	0	0
		(knee joint contained infected fluid).				
17.	0	0	0	0	+ viseral & parietal	+ viseral & parietal
18.	+	0	0	0	+	0
22.		(had not been killed).				
23.	0	+	+	0	0	0
47.	+	0	+	0	0	0
50.	+	+	+	0	0	0
54.	0	0	0	0	0	0
		Controls.				
57.	0	+	0	0	0	0
58.	+	0	+	0	0	0
44a.	+	+	+	+	0	0

Series II.

Tabulations of results, (continued).

No.	Lymph nodes.	Condition.	Seat of Injection.
10.	Prescap.	Good.	Thickened.
27.	Prescap.	Good.	Thickened.
39.	Prescap.	Fairly good	Thickened.
42.	Prescap.	Good.	Thickened & gritty.
44.	Prescap. no. bact.	Moderate.	Thickened & gritty.
17.	Prescap.	Good.	Thickened & gritty.
18.	Prescap. Pharyngeal Bronchial Mediastinal Hepatic.	Poor.	Thickened & gritty.
22.		Fair.	
23.	Bronchial Mediastinal	Good.	Thickened & gritty.
47.		Moderate.	Abscess.
50.	Mediastinal Hepatic Tracheal.	Fair.	Pin head tubercle.
54.		Good.	Gritty material.
57.	Bronchial Hepatic Tracheal Mediastinal	Good.	Gritty material.
58.	Gastric Pharyngeal Bronchial.	Poor.	Gritty material & tubercles.
44a.	Bronchial Illiac Mediastinal	Poor.	

Series III.

Eight animals were used in this series, two of which received two injections of the human vaccine, and four of which received two injections of the avian vaccine. The remaining two were used as controls.

Tabulations of injections.

No.	Date of 1st vac.	Nature of bac.	Dose in mg.	Date of 2nd vac.
38.	25/11/11.	Human.	10	19/1/12.
41.	25/11/11.	Human.	15	19/1/12.
21.	10/8/11.	Avian.	10	2/11/11.
48.	25/11/11.	Avian.	20	19/1/12.
53.	25/11/11.	Avian.	20	19/1/12.
55.	25/11/11.	Avian.	20	19/1/12.
28.	Controls (unvaccinated).			
29.	Controls (unvaccinated).			

Series III.

Tabulations of injections, (continued).

No.	Nature of bact.	Dose in mg.	Date of test inj.	Dose in mg.	Date of death.
38.	Human	30	30/7/13	30	3/11/13.
41.	Human	35	30/7/13	40	13/12/13.
31.	Avian	40	30/7/13	30	16/12/13.
48.	Avian	30	30/7/13	30	1/11/13.
53.	Avian	40	30/7/13	40	31/10/13.
55.	Avian	50	30/7/13	50	1/11/13.
28.			30/7/13	30	31/10/13.
29.			30/7/13	40	1/11/13.

Series III.

Tabulated results.

No.	Lung.	Liver.	Spleen.	Kidney.	Perito-neum.	Pleura.
88.	0	0	0	0	0	0
41.	0	0	0	0	0	0
21.	+	+	+	0	0	0
48.	+	0	0	0	+ parietal	0
53.	+	+	0	0	0	0
55.	+ (diaphragm & pericardium involved).	+	+	0	0	+ viseral & parietal
38.	+	+	0	0	0	0
29.	+	+	+	0	0	0

Series III.

Tabulated results, (continued).

No.	Lymph nodes.	Condition.	Point of injection.
88.	Prescap. Prepect. Coecal.	- Very good.	- Soft swelling.
41.	Bronchial. Mediastin. Tracheal Mesenter. Prescap.	- - Good.	- Soft swelling.
31.	Prescap. Bronchial	- - Fair.	Swollen.
48.	Prescap. Bronchial Mediastin. Pectoral	- - Good.	- - Swollen.
53.	Prescap.	- - Good.	- Swollen & gritty.
55.	Prescap.	- - Good.	- Enlarged & fibrous.
28.	Bronchial	- - Good.	- Enlarged & custard like.
29.	Prescap. Bronchial Prepect. Portal	- - Good	- Enlarged & fibrous.

Series IV.

This series consisted of twelve calves, five of which were injected with human, and five with avian vaccines. The remaining two were left as controls.

Tabulations of injections.

No.	Date of vac.	Nature of bact.	Dose in mg.	Date of test inj.	Dose in mg.	Date of Death.
61.	20/2/13	Human	10	21/6/13	50	3/12/13.
62.	20/2/13	Human	10	21/6/13	25	3/12/13
63.	20/2/13	Human	10	21/6/13	50	3/12/13
64.	20/2/13	Human	10	21/6/13	25	4/12/13
65.	20/2/13	Human	10	21/6/13	50	4/12/13
71.	20/2/13	Avian	10	21/6/13	50	4/12/13
72.	20/2/13	Avian	10	21/6/13	25	5/12/13
73.	20/2/13	Avian	10	21/6/13	50	5/12/13
74.	20/2/13	Avian	10	21/6/13	25	22/10/13
75.	20/2/13	Avian	10	21/6/13	50	5/12/13
82.	Controls (unvaccinated)			21/6/13	25	25/7/13
83.				21/6/13	50	4/8/13

Series IV.

Tabulated results.

No.	Lung.	Liver.	Spleen.	Kidney.	Perito-neum.	Pleura.
61.	0	0	0	0	0	0
62.	0	0	0	0	0	0
63.	0	0	0	0	0	0
64.	+	0 (pericardium affected).	0	0	0	+
65.	0	+	+	0	+ & omentum	+
71.	+	+	0	0	+	+
72.	0	0	0	0	0	+ costal
73.	+	0	0	0	0	+ costal diaphragm
74.	+	0	0	0	0	0
75.	+	+	0	0	0	+ costal pericardium
Controls.						
82.	+	+	0	+	0	+
83.	+	+	+	+	0	

Series IV.

Tabulated results, (continued).

No.	Lymph nodes.	Condition.	Point of injection.
61.	Prescapular Tracheal	Moderate	Enlarged, containing pus.
62.	Prescapular	Moderate.	Enlarged, dry and caseous
63.	Prescapular Mediastinal Prepectoral Pharyngeal	Moderate	Fibrous enlargement.
64.	Prescapular	Moderate	Enlarged, full of pus.
65.	Prescapular Bronchial Portal	Moderate	Enlarged, thick skin.
71.	Prescapular Bronchial P. mediastinal Pharyngeal Popliteal	Moderate	Enlarged, thick skin.
72.	Prescapular Post mediastinal	Moderate	Enlarged, thick skin and abscess.
73.	Prescapular	Moderate	Thick skin.
74.	Prescapular Bronchial Mediastinal Tracheal Mesenteric Prepectoral	Poor	Thick skin and caseous.
75.	Prescapular	Moderate	Thick skin & caseous.
82.	Prescapular Prepectoral Mediastinal	Very poor	Swollen & necrotic
83.	Mediastinal Spleenic Submaxillary Tracheal Mesenteric	Very poor	Swollen & necrotic

M'Faydean, in his conclusions, presented the following:

By intravenous injections of avian tubercle bacterium, it is possible to confer on healthy calves a markedly increased power of resistance to infection with the bacterium of the bovine type.

Such a method of vaccinating young cattle against tuberculosis involves little or no risk to the animal.

When vaccination of young cattle against tuberculosis is considered advisable, the avian bacterium should be preferred to the human in order to avoid the danger of infecting human beings with the bacteria that are present in the bodies of the vaccinated animals and passed out with their milk.

Himmelberger (3) of this laboratory, in a study of the transmissibility of avian tuberculosis, has been able to produce the disease in calves by feeding in milk macerated organs from a tubercular hen. He also tested the animals with avian tuberculin and was able to get a typical reaction.

Previous to this, he injected two rats, two rabbits and two guinea pigs subcutaneously with five cubic centimeters each of a potato broth culture of avian tubercle bacteria. One month later all were killed and upon autopsy the rats, guinea pigs and one rabbit showed no lesions, while the other rabbit showed an abscess at the point of injection in which tubercle bacteria could be demonstrated. It might be stated that the culture Himmelberger used had been cultivated upon artificial media for a number of years.

He also tried to infect rats by alternating pens of rats with pens of infected hens, but met with no success.

Along with his other experiments he tried to infect rabbits by injecting intravenously two centimeters of a suspension of macerated diseased organs. Upon autopsy the rabbits showed no lesions either microscopically or macroscopically.

The virulence of the human compared with the bovine tubercle bacteria has been studied at different times by a number of men and practically all have concluded that the bovine organism is the

more virulent.

Park and Krumweidd (4) in an extensive study along these lines, tested the virulence of the two organisms by making intravenous injections into both rabbits and calves.

Since only certain phases of their experiments have a direct bearing on our work, we will review only those portions which we believe are closely related to our studies.

The material used by them was usually taken from a growth on egg medium. It was removed by means of a sterile platinum spatula and transferred to smooth filter papers so as to remove all moisture. The weight of the mass was determined and the proper dilutions made so that one cubic centimeter of the suspension contained one milligram of the bacteria. Other dilutions were made so that one cubic centimeter of the suspension represented .01 milligrams. The rabbits used were weighed both before and after injection, with the exception that those receiving the human injection were weighed once in the interim.

Through their work the principal object they

had in mind was to produce progressive lesions and from their observations they were led to believe that lesions from virus of low virulence retrogressed; the number of lesions was found to be greater in those cases that died shortly after injection, than those that lived for a long period. Second, they did not make microscopical examinations of all lesions that showed no gross lesions, in as much as they thought that it would not give them absolute proof, unless a routine examination was made of the negative organs of other animals, as controls. Thus the number and location of the lesions was their index.

A generalized form of tuberculosis was produced in every case when .01 milligrams of bovine tubercle bacteria were injected intravenously. When using the human bacterium in the same amounts intravenously no disease was produced, never producing a generalized form. When one milligram was injected there was no generalization and as well no progressive lesions. When still larger doses were used death sometimes occurred but the lesions found could not be considered as the cause of death.

In a rabbit injected with .01 milligrams of bovine tubercle bacterium, the following lesions might be visible:

Lymph nodes.	Lungs.	Heart & adjacent pleura.	Spleen.	Liver.	Peritoneum & intestines.	Rib marrow.
auxillary. inguinal. bronchial. mediastinal. mesenteric. hepatic. spleenic.						

In cases of one milligram injections, a more acute type of the disease was produced; the general picture being the same but in most cases the gross lesions were small. In some cases it should be stated that death resulted from acute infection.

In cases of injections of .01 milligrams of the human type of tubercle bacteria, localized forms of the disease predominated chiefly in the lungs and less often in the kidney.

By injecting one milligram of the material the lesions were found to be about the same, possibly in some cases a little more marked; some generalization

was observed.

From the results they obtained they were able to set a standard in differentiating the human organism from the bovine by their virulence on rabbits, which was as follows:

When one milligram doses of the bovine organism are used:

1st. There is a tendency for the bovine virus to kill acutely without any signs of generalization.

2nd. If death is uniformly caused by a virus in less than thirty days and there is an absence of generalization in those dying in less than twenty days, the bacteria is bovine.

3rd. If a rabbit survives for fifty to sixty days with lesions only in the lungs or kidneys or even a few scattered lesions elsewhere, the virus is of the human type.

4th. If a rabbit dies within the first thirty to sixty days without showing the presence of generalization, it would be safe to draw the conclusion that it was due to the bovine tubercle bacteria.

A time limit was also set, when small doses were administered, which is as follows:

A rabbit surviving a milligram for sixty days or .01 milligrams for forty days and showing no signs of generalization was considered to have been infected by a human virus. If there was any tendency toward generalization with a large dose the smaller dose was also used.

In their summary they made the following statements:

The rabbit is the best animal for testing virulence for the diagnosis of type. The intravenous inoculation of .01 milligrams of culture is the best method of determining these differences. Any virus that is capable of producing progressive generalized tuberculosis with this dose of bovine type, the extent of the lesions being most important, the time of death being less important. All viruses incapable of causing lesions elsewhere, other than in the lungs or kidneys or in both are of the human type. Chance localization elsewhere is only occasional and not constant, being ruled out because a bovine virus is capable of producing a generalized

form of tuberculosis in every rabbit.

Virulence for Calves.

Much work has been done as to the virulency of the bovine and human organisms when injected into calves, but there has been an extreme variation of results as a whole.

In the work previously discussed, it would seem that the human and bovine organisms are each in a class by itself. The following review will show the effects produced when the same organisms are injected into calves.

All calves were tuberculin tested before being used.

In every case where a bovine culture was injected, a rabbit was given a dose from the same source, fifty milligrams of a culture being injected into the neck of the calf.

Eighteen strains of the human virus were used, and only two calves showed any signs of dissemination of the lesions. One calf showed doubtful lesions in the lungs which proved to be positive when injected into a guinea pig. Another calf showed typical lesions in the mesenteric lymph nodes, ma-

croscopically, but when material from them was injected into guinea pigs it failed to produce the disease. None of the lesions were extensive enough to be considered as generalized.

Fourteen strains from the bovine were used, eight of these being from human sources. Of these, six produced a generalized form of tuberculosis, resulting in death after a period of from twenty-three to seventy-three days. One calf showed a widespread form, which produced no ultimate effect upon the animal. Another was practically non-virulent which was shown by the control rabbit. In regard to those injected with the bovine virus, similar results were obtained.

Four caused progressive tuberculosis resulting in death in 20-90 days. One caused very slight tuberculosis but sufficient to influence the health of the calf. One was known to be non-virulent but was injected to compare with the control rabbits.

From their work with calves they summarized as follows:

When calves are to be used for the differentiation of types, early cultures should be selected,

there being one disadvantage to the calf method as the bovine culture gives small growth.

Parallelism with the rabbit is very close.

The Royal Commission on Tuberculosis (5) was instituted by appointments in 1901. The purpose of this commission was to report upon the following:

1. Is tuberculosis in man and animals one and the same disease?

2. Can man and animals be reciprocally infected with it?

3. Under what conditions if at all, does the transmissibility of the disease from animal to man take place and what are the circumstances favorable or unfavorable to such transmissibility?

In this report the first and second of these were dealt with.

Two parallel investigations were carried on at two different farms, using calves of the Jersey breeds from three weeks to five months old. In some cases adult animals of the same breed or of the shorthorn breed were used.

As to the source of the bovine virus used,

thirty lesions from naturally occurring tuberculosis in cattle were used. In introducing this virus into the cattle, two methods were employed; (1) feeding, (2) injecting into the tissue. Of the tissue injections, three methods were used, subcutaneous injections, intravenous injections, and intra mammary injections through the teat opening.

The effects produced by these injections may be summarized as follows: The bovine tuberculosis bacterium when introduced subcutaneously into the body of the bovine, whether in the form of emulsion or as a culture may produce; (1) fatal tuberculosis of generalized form, (2) a limited retrogressive tuberculosis, (3) or may show intermediate effects.

The amount of disease produced was found to rest upon certain factors, for example, the amount of material injected and the susceptibility of the animal.

The work done with the human bacterium consisted of securing material from sixty cases of human tuberculosis. Of these two groups were formed, being distinguished by the properties and character-

istics of the bacteria. A third group was formed which could not be classed under the first or second as it had properties of its own.

Group I consisted of fourteen cases, the viruses being obtained from either sputum or surgically removed tubercles from cervical glands, and primary cases of abdominal tuberculosis. All the cases of abdominal tuberculosis were obtained from children.

Group II consisted of forty cases of different origin. In some cases the material consisted of material removed surgically, eight from mesenteric lymph nodes removed after death, ten from lungs and bronchial glands, or from a diseased kidney, one from a tubercular testis and the remainder were taken from diseased joints or bones.

The effect of the bacteria of the first group, when injected into calves was found to be the same as the effect of the bovine bacteria when injected into calves.

The bacteria of group II, was found to be far less virulent in large doses. It failed to produce progressive generalized tuberculosis in the bodies

of rabbits and calves. When subcutaneous injections were made it was found that the resistance of the body tissue was greater than the virulence of the organism, thus setting up a retrogressive form of tuberculosis or none at all.

Group III was not placed under this head because the organisms produced like results when injected into animals but were grouped for the sake of convenience as they had individual characteristics.

Their Conclusions.

There can be no doubt that in a certain number of cases the tuberculosis occurring in the human subject, especially in children, is the direct result of the introduction into the human body of the bacterium of bovine tuberculosis; and there also can be no doubt that at least in the majority of these cases the bacterium is introduced by the consumption of infected cow's milk. Cow's milk containing the tubercle bacteria of bovine source is clearly the cause of tuberculosis of fatal termination in man in many cases.

Of the sixty cases of human tuberculosis in-

vestigated, fourteen belonged to Group I, that is to say, they contained the bovine virus. Instead of taking all forty of these cases, they confined themselves to those cases of tuberculosis in which the bacteria were apparently introduced into the body by way of the alimentary tract. Of the total sixty cases investigated, twenty-eight possessed clinical history indicating that the bacteria were introduced by way of the alimentary tract. Where cervical glands were studied, a large percent were found to be of alimentary origin.

These facts indicate that a very large percent of cases of tuberculosis in man contracted by ingestion is due to the tuberculosis bacterium of the bovine source.

A very considerable amount of disease and loss of life in man, especially among the young must be contributed to the consumption of cow's milk harboring the tubercle bacteria from diseased bovines. The presence of the organism can be detected in the milk though with some difficulty if the proper means be adopted. Such milk should never be used as food.

They further state, that from a health standpoint, more stringent laws should be enforced as to the sale and consumption of milk.

Technique of Our Experiment.

The cultures used were grown on glycernated potato and carrot slants. After the organism had grown luxuriantly they were removed with a sterile platinum loop and placed on sterile filter papers to dry. As nearly as possible all the moisture was removed. It was then weighed and the proper dilutions made in sterile salt solution so that one cubic centimeter of the suspension contained .001 gram of the dried avian organism.

The rabbits used were all weighed before injecting and again at death, so that the loss or gain in weight might be determined. There were a few rabbits injected with .01 gram of the suspension, but it has been our experience, as well as that of Park and Krumwiede, that when a large dose is injected there will develop an acute form of the disease and usually death will result before any macroscopic lesions develop.

In this series which we will designate as series A, eighteen rabbits were subjected to intravenous injections of a suspension of avian tubercle bacteria.

Two were injected with a macerated diseased spleen of a hen, two with macerated bovine lesions, and two with a suspension of material from a human culture.

Some of the rabbits were autopsied in thirty days, when in a moribund condition, while others were allowed to live for four months. If no gross lesions were found at death a microscopic examination of the material was made.

Tabulation of Results.

Culture & No. amt. inj.	No. days lived	Loss in weight.	Post Mortem Findings.
1. N.D. .01 gm.	30	323 gms.	No lesions found on macroscopical examination. Many tubercle bacteria found microscopically in liver, lungs, spleen & kidney as well as adjacent lymph nodes.

Tabulations of Results.

No	Culture & amt. inj.	No days lived.	Loss in weight	Post Mortem Findings.
2.	N.D .01 gms.	28	410 gms.	Lesions in apices of both lungs. Bacterium microscopically in liver, spleen and adjacent lymph nodes.
3	Mixed Wis. .01 gms.	19	350 gms.	Tubercles in liver and spleen. Found bacteria in lungs. Kidneys normal.
4	Mixed Wis. .01 gms.	138	1180 gms.	Very badly emaciated, hind parts paralized. No macroscopical lesions. Bacteria were found in lungs, liver, spleen, kidney and adjacent lymph nodes. Femoral-tibial joint involved.
5	M. .01 gms.	42	490 gms.	Fair condition. Tubercles found in liver, spleen, and smears from lungs showed many bacteria.
5a	13 .001 gms.	109	715 gms	Lesions in lungs, liver spleen, kidney. Peritoneum slightly involved.
4a	13 .001 gms.	106	785 gms.	Lungs, liver, spleen and kidney badly involved. Parietal peritoneum studded with small tubercles in region of kidney & stomach.
13	75 .001 gms.	36	692 gms.	Both lungs badly involved with small tubercles. Spleen, kidney and adjacent lymph nodes, involved.

Tabulations of Results, (continued).

No	Culture & amt. inj.	No days lived.	Loss in weight.	Post Mortem Findings.
6	13 .001 gms.	144	565 gms.	Lungs, spleen, hepatic and mesenteric lymph nodes involved.
14	75 .001 gms.	Killed on 114th day.	787 gms.	Lungs present many small tubercles. Spleen normal. Kidneys and scapro-humoral joints involved.
15	13 .001 gms.	62	792 gms.	Macroscopically, peritoneum, kidney, liver, spleen; microscopically, lungs.
19	13 .01 gms.	30	760 gms.	Mesentery, intestines, liver, spleen, kidneys and lymph nodes.
31	75 .01 gms.	29	1010 gms.	No macroscopic lesions except in hepatic lymph nodes. Found tubercle bacteria microscopically in lungs, hepatic glands, kidney and spleen.
32	13 .001 gms.	46	310 gms.	Lungs, liver, spleen, kidney, all showed lesions.
18	Mixed 75 & N.D .001 gms.	16	630 gms.	Liver, lungs, thoracic wall and thoracic pleura, parietal peritoneum, spleen and lungs.
3a	13 .001 gms.	90	342 gms.	Lungs, liver, spleen, mesentery and peritoneum.
1a	13 .001 gms.	72	470 gms.	Lungs, thoracic wall, peritoneum, spleen and liver.

Tabulations of Results, (continued).

No	Culture & amt. inj.	No days lived	Loss of weight.	Post Mortem Findings.
23	13 .001 gms.	114	248 gms. gain.	No lesions macroscopically or microscopically.
A.	Bovine tissue	Killed on 45th day.		Milary tuberculosis of liver, spleen, kidney, lungs and diaphragm.
B.	Bovine tissue	Killed on 45th day.		Lesions in lungs, liver, spleen and peritoneum.
C.	Human culture .001 gms.	Killed on 60th day.		Lesions in the apex of left lung. No other evidence of infection.
D.	Human culture	Killed on 60th day.		Several small tubercle foci in both lungs.
E.	Avian tissue	Killed on 45th day.		Lesions in liver, spleen and kidney.
F.	Avian tissue	Killed on 45th day.		Lungs, peritoneum, spleen, kidneys and diaphragm.

From these results it can be plainly seen that the rabbits receiving intravenous injections of avian tubercle bacteria developed a generalized form of tuberculosis.

The rabbits receiving the injections of a diseased macerated spleen from a hen developed a like form of the disease.

The two rabbits receiving the injections from the diseased bovine tissue, developed a very severe form of generalized tuberculosis which resembled that from produced by the avian organism.

In the case of those injected with the human organism, a localized form was the result.

Series B.

This series consisted of fifteen rabbits injected with cultures of the avian organism from different sources. Two of the cultures were from the North Dakota and Wisconsin Experiment Stations, while the third was isolated from the spleen of a hen, by the author. They were grown on glycernated potato and carrot slants.

In series A we had great difficulty in making some of the intravenous injections in as much as the suspensions contained so many clumps. In this series, we devised a method that worked more satisfactorily and did away with all previous difficulties im injecting.

The growth was removed from a slant as before, with a sterile platinum loop and placed between two sterile filter papers to remove all moisture. To

the mass was added a quantity of sterile NaCl. solution making a concentrated suspension. This was placed in the shaking machine which was allowed to run for about three or four hours. The suspension was then filtered through a small piece of cotton to remove all the large clumps so that it would pass easily through a fine hypodermic needle. This left a heavy, milky suspension which was weighed. The weight of the Na.Cl. solution was subtracted from the weight of the suspension which gave the weight of the bacteria. The proper dilutions were made so that each cubic centimeter by weight contained .001 gram of the avian culture or the dose for a rabbit.

All rabbits injected were females, the injections being made intravenously in the right ear.

Tabulation of Series B.

No.	Amt. of inj. & culture	Loss in weight.	No days lived.	Autopsy Findings.
1.	.001 gms.	570 gms.	32	Much emaciated liver, lungs, spleen and adjacent lymph nodes affected.

Tabulation of Series B, (continued).

No.	Amt. of inj. & culture	Loss in weight.	No. days lived.	Autopsy Findings.
2	.001 gms.	770 gms.	38	Lesions in liver, spleen, lungs. Microscopical examination of the kidneys showed numerous tubercle bacteria.
3	.001 gms.	380 gms.	38	Liver, spleen and kidney affected.
4	.001 gms.	715 gms.	48	Much emaciated lesions found in the lungs, liver, spleen and adjacent lymph nodes. Kidneys showed a few pinhead lesions.
5	.001 gms.	590 gms.	39	Lungs, liver, spleen and adjacent lymph nodes affected.
6	.001 gms.	400 gms.	70	Lungs, liver, and adjacent lymph nodes affected. No bacteria were found in other organs.
7	.001 gms.	430 gms.	70	Lesions in lungs, liver, spleen and adjacent lymph nodes. Kidneys showed no tubercular bacteria, microscopically.
8	.001 gms.	1330 gms.	70	Very poor in condition. Lesions in lungs, liver and adjacent peritoneum; spleen studded with large tubercles.

Tabulation of Series B, (continued).

No.	Amt. of inj. & culture	Loss in weight. grams.	No. days lived.	Autopsy Findings.
9	.001 gms.	80	76	Found slight lesions in lungs and liver. Microscopically, no bacteria were found in other parts.
10	.001 gms.	1090	31	Much emaciated and unable to eat; lesions found in lungs, liver, spleen and adjacent lymph nodes. Kidney showed many bacteria microscopically.
11	.001 gms.	1140	37	Much emaciated; breathing labored at time of death. Autopsy showed a consolidation of a greater part of both lungs as well as the parietal pleura; liver, spleen and both kidneys showed many lesions.
12	.001 gms.	170	71	Lungs and liver showed a few lesions. Mediastinal lymph nodes involved; no bacteria were found in other organs, microscopically.
13	.001 gms.	5 gain.	84 killed.	In good condition; a few bacteria were found in the lungs.
14	.001 gms.	415	41	Fair condition; lesions in lungs, liver, spleen and adjacent lymph nodes; microscopically found bacteria in kidneys.
15	.001 gms.	500	54	Lungs, liver, spleen and adjacent lymph nodes were affected; condition fair; microscopically, no bacteria were found in the kidneys.

Series A.

Tabulations of Parts Affected.

Rabbits showing lesions or the microörganisms microscopically in five groups of organs.

No.	Lungs	Liver	Spleen	Kidney	Pleura.	Peritoneum	Lymph-nodes	Diaphragm	Mesentery.
4	+	+	+	+	o	o	+	o	o
				(femoro-tibial joint)					
A	+	+	+	+	o	o	+	+	o

Rabbits showing lesions or the microörganism microscopically in four groups of organs.

L	+	+	+	+	o	o	+	o	o
5a	+	+	+	+	o	+	o	o	o
4a	+	+	+	+	o	+	o	o	o
15	+	+	+	+	o	+	o	o	o
32	+	+	+	+	o	o	o	o	o
F	+	o	+	+	o	+	o	+	o

Rabbits showing lesions or the microörganism microscopically in three groups of organs.

18	+	+	+	o	+	+	o	o	o
2	+	+	+	o	o	o	+	o	o
3	+	+	+	o	o	o	o	o	o
5	+	+	+	o	o	o	o	o	o
13	+	+	+	o	o	o	o	o	o
6	+	+	o	o	o	o	+	o	o

Series A.

Tabulations of Parts Affected, (continued).

No.	Lungs	Liver	Spleen	Kidney	Pleura.	Perito-neum	Lymphnodes	Diaphragm	Mesentery.
14	+	o	+ (scapulo-humoral joint)	o	o	o	o	o	o
19	o	+	+	+	o	+	+	o	o
31	+	+	+	o	o	o	+	o	o
18	+	+	+	o	+	+	o	o	o
3a	+	+	+	o	o	+	o	o	+
La	+	+	+	o	o	+	o	o	o
Bb	+	+	+	o	+	+	o	o	o
E	o	+	+	+	o	o	o	o	o
	Rabbits showing lesions in one tract.								
C	+								
D	+								
23	Non infected.								

Series B.

Rabbits showing lesions or the microorganism in four groups of organs.

2	+	+	+	+	o	o	o
4	+	+	+	+	o	o	+
10	+	+	+	+	o	o	+
11	+	+	+	+	+	o	+
14	+	+	+	+	o	o	+

Series B.

Tabulations of Parts Affected, (continued).

No.	Lungs	Liver	Spleen	Kidney	Pleura	Peritoneum	Lymph-nodes	Diaphragm	Mesentery.
Rabbits showing lesions or the microorganism in three groups of organs.									
1	+	+	+	o	o	o	+	o	o
3	o	+	+	+	o	o	o	o	o
5	+	+	+	o	o	o	+	o	o
7	+	+	+	o	o	o	+	o	o
8	+	+	+	o	o	+	o	o	o
15	+	+	+	o	o	o	+	o	o
Rabbits showing lesions or the microorganism in two groups of organs.									
6	+	+	o	o	o	o	+	o	o
9	+	+	o	o	o	o	o	o	o
12	+	+	o	o	o	o	+	o	o
Rabbits showing lesions or the microorganism in one group of organs.									
13	+	o	o	o	o	o	o	o	o

+Indicates that this part was found to be infected either microscopically or macroscopically.

Assuming that when three tracts are involved a diagnosis of progressive generalized tuberculosis can be made, we can say that we have produced the above form in 82.5 percent of the cases under our observation.

In following the work of M'Faydean, some very interesting comparisons can be made of the pathogenesis of the human, bovine and avian tubercular bacteria.

In series I, calves 6 and 8 received but one injection of the human vaccine before being treated with the bovine test injection. Both calves developed a very severe form of tuberculosis which was detected on autopsy. Seven calves received the avian vaccine of which five received the second injection before being subjected to the bovine test inoculation. Upon autopsy it was found that they had all developed as serious a form as those injected with the human organism. The two controls were found to have a generalized form, being badly emaciated.

In series II, there were fifteen calves used of which five were subjected to the human vaccine,

seven to the avian and the remaining three were used as controls.

It would seem that the avian organism was far more virulent than the human organism when injected into calves intravenously or that more protection against the bovine organism was conferred by the human vaccine than by the avian.

A large percentage of those receiving the avian vaccine were very seriously infected, having a number of organs and tissues involved, while in the case of the five receiving the human vaccine only one was seriously affected and that was localized in the lungs.

The first control calves were in fair condition, having lesions in the lungs, adjacent lymph nodes and the mesemteric lymphatics. The remaining two were badly affected, having developed a generalized type of the disease.

In series III, there were eight calves used, two of which received two injections of the human vaccine, and four received two of the avian. The remainimg two were control calves.

The two receiving the human vaccine were in

good health and showed only a few small lesions in some of the lymph nodes. The four receiving the avian vaccine showed lesions in the lymph nodes and one or more of the vital organs, three of these being of the generalized nature. Both of the calves were in good condition, calf 28 having developed a localized form while calf 29 developed a generalized form of the disease. This series would lead one to believe that the avian organism was more virulent than the human and that there existed a relation as regards pathogenesis to that of bovine origin.

Calves receiving human organisms, localized form 100%.
Calves receiving avian organisms, localized form 25%.
Calves receiving avian organsims, generalized form 75%.
Calves receiving bovine test injection, generalized form 100%.

(This is further evidence of the great virulence of the bovine type).

Series IV.

In table 4 there were twelve calves used of which five received one injection of the human vaccine, and five received one injection of the avian

vaccine. The remaining two were unvaccinated controls.

Of those receiving injections of the human vaccine, three had slightly infected lymph nodes of different regions, one, infection of the lungs and adjacent pleura, while the fifth developed a generalized form.

Of those receiving the avian vaccine, all but one had infection of some vital organ, while calf 11 had a generalized form. Control calves 82 and 83 were both in poor condition and had a very severe form of generalized tuberculosis.

From the foregoing it can be plainly seen that there was infection in more vital organs and more forms of generalized tuberculosis by using the avian vaccine than by using the human.

In our experiments we have found that a large percent of the cases of progressive, generalized tuberculosis had lesions either in the lungs, liver or spleen, but often times showed other infected foci in the different parts of the body.

Most of the cases of miliary tuberculosis, both in series A and B, were cases of short dura-

tion, death usually occurring before there was much emaciation or any outward clinical symptoms. In series A, rabbit A, injected with material from a diseased lymph node of a cow , was in very poor condition at death; but since the weight of the material injected as well as that of the rabbit was not taken, an exact determination of the virulency can not be estimated.

Of those in series B that showed an involvment of two tracts, six were considerably emaciated, while rabbits 9 - 12 were apparently in good health at death.

Number 13 of series B was in good health at death (killed), gaining in weight during the experiment.

It was found that those rabbits having an infection of five tracts died within a period of from twenty to twenty-five days either of an acute infection or miliary tuberculosis.

In studying the effects of the different organisms we found that there was no great variation in the virulence of the different cultures as to the number of organs affected but it was found that

certain cultures would produce a diseased condition in a shorter period of time than others. In studying the different organisms as to their pathogenic effects the individual tolerance of the rabbit must be considered, as for example, rabbit 23 in series A and 6, 9, 12 and 13 of series B did not develop a serious form of the disease when kept under the same condition as those which were in a more or less morbid condition at death.

Description of Plates.

The material used for sections was obtained from the rabbits of series A. A number of sections were made and part of them were stained with heated carbol-fuchsin, then counterstained with methylen blue, while the remainder were stained with hematoxylin and eosin to show the pathologic changes that had taken place in the tissues.

The type of the disease produced was found to be somewhat variable in the different animals and the pathological changes in the different organs were uniform.

As was previously stated, those animals receiving large doses developed an acute form of the di-

sease and many times showed no macroscopic lesions at autopsy with the exception of now and then a hemorrhage of some part of the body. But in the rabbits that received the smaller doses there was no difficulty in finding lesions either in one or more of the organs.

The animals will first show signs of emaciation and loss of appetite. In some cases digestive disturbances were noticed and as the disease progressed the lung disturbances were evident from the increased respirations.

Those animals that died of the chronic form of the disease showed in many cases the miliary form of tuberculosis, most noticeable either in the liver or spleen.

Natural Infection may take place by one of the following methods: Ingestion, Inhalation or Wound Infection, but it is supposed that in most cases infection takes place by the first method.

After entering the body the organisms are disseminated through out the body either by the blood or lymph streams and are finally deposited in the tissue from the capillaries.

The number of tubercle bacteria that can be found in the different lesions when examined microscopically, is extremely variable.

The location of the organism is not always constant but depends upon a number of factors such as the organs affected and the extent and location of the lesion. Where there is an accumulation of epithelioid cells the bacteria may be contained within the cell body, but if there is a considerable amount of cell necrosis taking place they may be set free and will be found in the intercellular spaces. They appear to grow and spread within the lesion by direct growth from cell to cell with the aid of the ameboid movement of the cell.

Liver.

It is quite evident that infection of the liver takes place through the hepatic artery and portal vein. The lesions may be located in any portion of the organ but are more often seen in the region of the portal vein. The chains of liver cells will be more or less destroyed by the formation of epithelioid cells, Figure I. Some of the cells may show the presence of necrosis. Figure II

shows a large area of necrosis and the presence of many tubercle bacteria.

Spleen.

Infection of the spleen takes place through the blood streams and it is often associated with generalized tuberculosis. Often times the organ will be much enlarged and show areas of extreme hypodermia with an accumulation of epithelioid cells which will be easily detected upon microscopical examination. The Malpighian bodies are the last to become infected but finally fail to resist the invading organisms resulting in a proliferation of epithelioid cells. Figure III shows a section of the spleen studded with tubercle bacteria.

Lungs.

Infection takes place either through the blood or respiratory tract and in some cases the lymphatics. The type of disease produced may vary somewhat. Acute miliary tuberculosis is one of the more frequent as well as serious forms. The tubercle bacteria probably reach the tissue by way of the blood streams and in this way are disseminated throughout the organ.

In chronic tuberculosis the first lesions either arise from the blood or through the air passages, occasionally through the lymphatics. There will be first noticed a proliferation of epithelioid cells, later necrosis and in a number of cases calcification. Softening of a tubercle lesion is quite frequent, often times being due to the invading material. The softened material may discharge into a bronchus and produce caseous pneumonia or into a blood vessel and cause an acute form of generalized tuberculosis.

Regeneration of connective tissue may take place around a tubercle due to the organization of the fibrin produced as a result of the inflammation. Extensive fibrinous deposits are quite common in tuberculosis lung. Figure IV. shows an extensive diseased area filled with necrotic lung tissue and endothelial cells.

Kidney.

The types of tuberculosis produced in the kidney are not always the same. The three following types are most often found; maliary tuberculosis; tuberculosis infarcation and tuberculosis nephritis.

Miliary tuberculosis is more often seen than the other two forms, but it is not as frequent as miliary tuberculosis of the other organs. The lesions may first be seen in a glomerulus or in the capillaries between the tubercles. There will be noticed an area with an accumulation of epithelioid cells which later will change into areas of necrosis as the vessels are occluded. In cases of kidney infection the animal will soon die as it is quite likely that the primary seat of the disease is in some other organ.

Tuberculous infarcation is a form of tuberculosis often seen in the kidney and results from infection from an artery by which the tubercle bacteria are distributed to the smaller vessels of the main artery.

Tuberculous nephritis is a more chronic form of tuberculosis and is due to the organisms gaining entrance into the pelvis of the kidney thus producing pyelitis. The tubules will later be infected leading to a spread towards the cortex of the organ. Numerous abscess formations will be found which will later result in necrosis and ulceration.

Lymph nodes.

Of the lymph nodes most frequently infected are the cervical, mediastinal, bronchial, hepatic and mesenteric although others may be involved.

Infection likely takes place more frequently through the lymphatics, less often through the blood.

The bacteria are probably disseminated by means of the ameboid movement of the epithelioid cells. The organisms that are carried to the produce a generalized form of tuberculosis while those that remain in the sinuses cause an accumulation of leukocytes which fill and destroy the vessel. The miliary lesions usually spread and in a short time will form one solid mass by the fusing of the numerous tubercles.

Conclusions.

It would seem unsafe to use the living avian organism in preparing a vaccine for cattle.

It is usual to produce in rabbits a progressive form of generalized tuberculosis by intravenous injections of the avian tubercle bacteria.

In larger doses than .001 gm. intravenously

an acute form of the disease will be produced causing the death of the rabbit in a few days.

From the results we have obtained it would lead one to believe that a similarity exists between the avian and the bovine type of the tubercle bacteria as regards their virulency.

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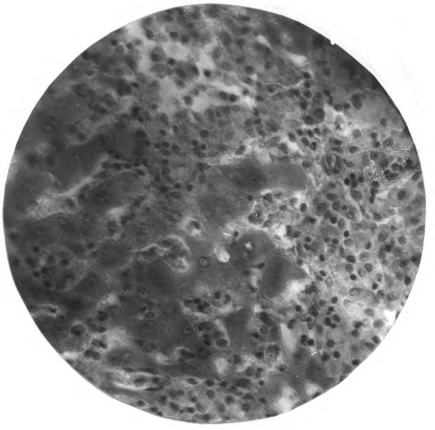

Fig. I.

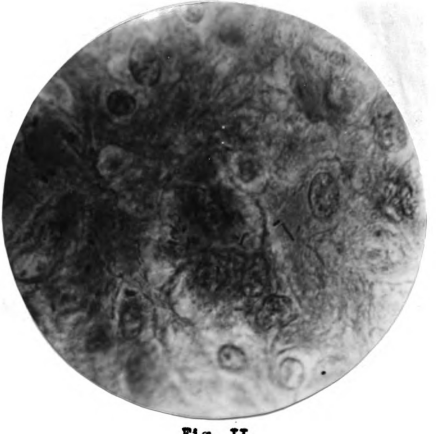

Fig. II.

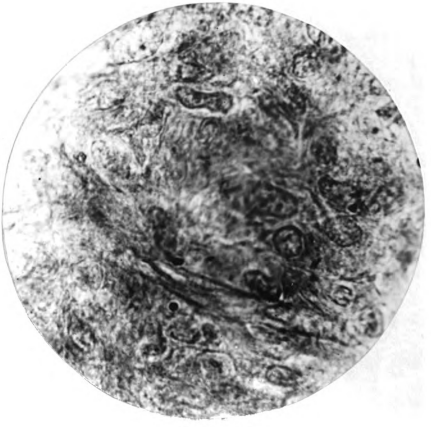

Fig. III.

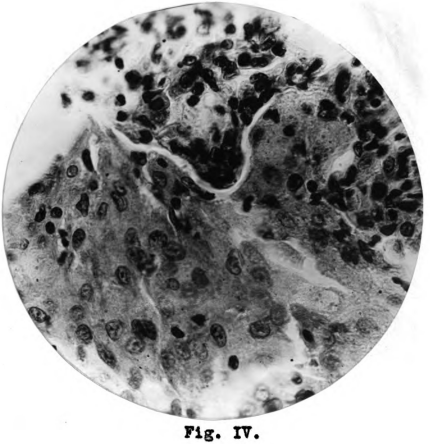

Fig. IV.

Mosher
Studies on the patho-
genesis of the avian
tubercle bacteria