

A STUDY TO DETERMINE THE PRESENCE OF MYCOBACTERIUM TUBERCULOSIS AVIUM IN THE EGGS OF INFECTED BIRDS THESIS FOR THE DEGREE OF M. S. Robert J. Biggar 1932 Bulling - Disi and Myerbaatinum tuberculoseo artur

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THESIS 11.5

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Submitted to the Faculty of the Michigan State College in partial fulfillment of the requirements for the Degree of Master of Science

by

Robert J. Biggar 1932.

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Introduction

The poultry industry has risen to a position of vast importance in the United States. Consequently, the problem of avian diseases has increased proportionally. Due to the modern means of transportation, the interchange of poultry between widely separated localities has been a great factor in widening the spread of diseases which otherwise might have remained more localized.

Avian tuberculosis is generally recognized as one of the major diseases of poultry. A survey by Mohler (1) showed about 5.7 per cent of 16,500,000 chickens in 38 states to be infected. In the Mid-West this figure would undoubtedly run higher.

Additional importance is given the problem by the finding of infections of other farm animals, such as cattle and swine, by this variety. It is interesting to note that Löwenstein (2) says that the human infection by the avian type is much more prevalent than commonly supposed, this statement is affirmed by other workers in this field. Klimmer (3) reports 35 cases of tuberculosis in man due to infection by the avian type.

It is common knowledge that this disease is rapidly transmitted from an infected fowl to others of the flock by the ingestion of <u>Mycobacteria</u> <u>tuberculosis</u> present in the droppings of the diseased bird. It readily follows that if one fowl infected with tuberculosis is introduced into a flock of birds, the entire flock is exposed and extensive infection throughout is apt to be very rapid. Thus it behooves those studying this disease to ascertain every possible mode of dissemination and to minimize every means to the point of elimination.

It is with this in view that we have undertaken this study to determine the presence of the <u>Mycobacterium</u> <u>tuberculosis</u> in the eggs of infected fowls, and to obtain sufficient evidence to allow a fairly accurate estimate of the danger to be expected from this source. Further studies on the transmission of the organism to the chick from the egg will be reported from this Experiment Station at a later date. •

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Review of Literature

The work done to determine the incidence of the presence of <u>M. tuberculosis</u> in the eggs is insufficient to allow a conclusion to be made as to what extent this presence attains. About 50 per cent of the writers claim that "there is no experimental evidence to show that tuberculosis is transmitted from the hen to the chick through the egg"(4). In the work of Van Es and Schalk (5) the following statement is made: "It seems thus that the transmission by means of the egg must be given consideration, although the data are not sufficiently numerous to enable us to correctly estimate the extent of the danger".

The first workers on avian tuberculosis to point out the possibility of a congenital transmission through infection of the embryo were Kock and Rabinowitsch (6). Since then many statements both pro and con have been made by various investigators. Gartner (7) in his work with artificially infected canaries found virulent organisms in two out of nine eggs. Higgins (8) did a considerable amount of work with eggs from tuberculous birds. He succeeded in deomonstrating bacilli in three out of six eggs by microscopic examination. This figure is considerably higher than the writer has obtained using this same method of examination. Gallagher (9) reports from his findings that "eggs of diseased birds frequently contain the microbes". By injection of egg material into guinea pigs. Mohler and Washburn (10)

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proved two eggs to contain virulent organisms. Artault (11) likewise was able to produce infection in rabbits with two out of twenty-five eggs. He claims the eggs were "undoubtably infected in the oviduct" but no autopsy findings were published with his report. The most positive statement that has been made seems to be that given by Fitch, Lubbehusen, and Dikmans (12) after examining a total of 876 eggs from tuberculous hens. They state: "It would be safe to conclude from these results that less than one per cent of eggs from tuberculous fowls actually contain living tubercle bacteria". Although this figure is exceedingly low, it shows conclusively that the organism may be present in the egg. It is quite probable that if a suitable technique could be developed, a higher percentage would be obtained. In another extensive study of the question by Fitch and Lubbehusen (13), they conclude: "We must view the danger of transmission of tuberculosis through naturally infected eggs of little practical consequence".

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Statement of Problem

After going over available literature on the subject of the transmission of the <u>Mycobacterium</u> <u>tuberculosis</u> from infected birds to the eggs, one observes that the proof is not definitely established, and that each worker interprets the results of his research in a different way. It is with this in mind that the writer has conducted the experiments here in set forth. Two objectives have been in view; first, the isolation of the bacilli directly from the egg; and secondly, the finding of tubercular lesions in the organs of the reproductive system.

The method of procedure used in this experiment differs from those of other workers on the problem, in that the fowls used for the work were all obtained from healthy stock and were infected artificially. The author believes this point to be of paramount importance as it gives the worker an opportunity to ascertain what stage of advancement the disease has attained when he may expect to find the <u>M. tuberculosis</u> to be transmitted to the egg. There is the possibility that the stages of the disease may differ as to the distribution of the organisms.

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Experimental

Twenty healthy, egg producing hens were selected. leg-banded, and trap-nested: to these were added two cocks. On the eleventh of January the 20 hens were divided into three groups. Seven hens were given one intravenous inoculation; seven were given intramuscular inoculation. the injection being made into the muscles at the right side of the breast: and the remaining six were given inoculations per os. Those receiving the inoculations through the oral route were given doses of varying sizes, ranging from two to five cubic centimeters. Some of these hens were inoculated as often as five times. at two day intervals. In giving the per os inoculation, great care was taken to pass the catheter down the esophagus to the gizzard to avoid a primary lung infection. However, in spite of this precaution, one of this series developed a large caseous lesion in the right lung.

The material used for intravenous and intramuscular injection was a preparation made by crushing in sterile saline solution, tubercles cut from a heavily impregnated liver of a naturally infected hen. The material to be administered per os was prepared by emulsifying with sterile saline the whole liver of a severe case of miliary tuberculosis in a bird brought into the clinic for autopsy.

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On the nineteenth of January, or eight days after injection, the entire group was given a tuberculin test. At this time all birds gave a negative reaction at 24 and 36 hours, thus giving satisfactory evidence that no previous infection existed. Four weeks after the injection of the tuberculous material, the flock was again given the tuberculin test. The results of this test are shown in Table 1.

Table 1.

	· · · · · ·				
Mode of injection	No.	tested'	No. po	sitive'	% reactors
Intravenous	1 1 1	7 1 1	0		0
Intramuscular	1 (1	5 I I	4	: T	66.66
Per os	<u> </u>	<u>; </u>	1	1	16.66

At the end of the sixth week with the exception of two birds, the entire flock reacted positively to the tuberculin test. These two birds never gave positive reactions to the test, although on autopsy one of the birds was found to have lesions in the liver and spleen, stained smears of which showed the tubercle bacilli.

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Three weeks after injection, the eggs of the hens were saved for examination. At first the eggs were incubated at 37°C. for three days to allow the <u>M.</u> <u>tuberculosis</u> to multiply. Later this practise was discontinued as other organisms present in the eggs were thought to possess a much greater rate of reproduction, thus counteracting the value derived from allowing the <u>M.</u> tuberculosis to multiply.

The technique employed in examining the eggs is adopted from that used by Beller and Henninger (14). The egg was first thoroughly cleaned and the shell disinfected by immersing in Chandler's Colloidal Iodine for three minutes. The egg was then placed on end in a small beaker and the tips flamed and opened aseptically with sterile forceps. The white and yolk were then blown into a sterile Erlenmeyer flask containing a small amount of one per cent NaHCO3 solution. This served the dual purpose of homogenizing the egg material and of reducing the number of gram negative organisms that might be present. After thoroughly homogenizing the yolk and albumin, the volume was made up to 100 c.c. with sterile redistilled water. The material was then shaken thoroughly and transferred into a 100 c.c. centrifuge tube and centrifuged for one hour and fifteen minutes at a rapid speed. The supernatant liquid was then poured off. Two smears were made

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from the sediment of each egg; the residue was then resuspended in six per cent HCL. This solution of acid was weak enough to allow the <u>M. tuberculosis</u> to remain uninjured but strong enough to destroy any other organisms present. The resuspension was then cultured on one slant of Petroff's egg medium and on one slant of Dorset's egg medium. The tubes were aseptically plugged, covered with tin-foil, and placed in a horizontal position in an incubator at 27°C. The smears made from the residue were stained with the Ziehl-Nielson's stain, using Loeffler's Methylene Blue as a counter stain, and examined under the oil immersion lens.

All eggs examined the first two weeks, or until the beginning of the fifth week after injection, were negative. At the end of ten weeks the slants cultured from these eggs showed no growth, neither did the smears from the residue show any acid-fast organisms.

The fifth week after infection, the smears made from the eggs were noticed to contain a few acid-fast granules. These granules were about eight-tenths of a micron in size, possessing a coccoid shape. They were not regular enough in form to be considered spores. It happened that at this time bird No. 7758 died. On autopsy, no lesions were found. There was, however, a slightly rough depressed area on the ventral side of the liver. Smears made from this area showed no typical organisms but showed many of these acid-fast granules.

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In the light of the work of Kahn (15) of Cornell University in which he reports his work and states his theory regarding the life cycle of M. tuberculosis, it was suspected that these might be similar to the granules described by him. With this in mind. scrapings of this area were suspended in sterile saline solution and two cubic centimeters were injected into the peritoneal cavity of a pullet free from tuberculosis. After six weeks, the bird was autopsied and lesions were found in the liver and mesentery from which typical tubercle bacilli were observed in stained smears. It is possible that the lesions may have been caused by the regular rod-shaped bacilli which might have been present in the liver. The writer, however, believes that if this were true the bacilli must have been very few as stained smears failed to reveal them on repeated examination.

Egg material containing these acid-fast granules was injected into the peritoneal cavity of a pullet, guinea pig, and rabbit. The inoculation was made with three cubic centimeters of a heavy suspension. The results of the inoculation are tabulated in Table 2.

TUCTO NO	T	a	b	1	e	2.
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Bird No.	' Date	Weeks after	Pullet	Rabbit	Guinea pig
4590	'Mar. 2	7	no lesions	no lesions	no lesions
7173	'Mar. 10	8	small lesions*	small lesions*	no lesions
4670	'Mar. 10	8	no lęsions	no lesions	no lesions

* Smears of lesions contained acid-fast bacilli.

Explanation of Table 2.

In the first column, the numbers are those of the birds from which the eggs to be tested were taken.

Column 2 gives the dates on which the $e_{\ensuremath{\mathcal{C}}\ensuremath{\mathcal{G}}\ensuremath{\mathcal{G}}\ensuremath{\mathcal{C}}\ensuremath{\mathcal{$

Column 3 gives the number of weeks after the injection before the autopsy.

Columns 4, 5, and 6 show autopsy findings.

While the results of these inoculations were positive in only 33.33 per cent of the tests, they nevertheless give direct proof of the presence of the <u>M. tuberculosis</u> in the egg, either in the rod-shaped form or as granules. Undoubtedly three cubic centimeters of the suspension was insufficient to be infective, and a higher positive result might have been attained using larger quantities. It is interesting to note, at this point, that only six of the twenty hens laid eggs in which the acid-fast granules could be found. In each of these cases, the acid-fast granules were observed in more than one egg, while the eggs of the other birds did not contain them. Four eggs from tuberculosis-free hens were artificially inoculated with the <u>M. tuberculosis</u> at various times throughout the experiment to serve in checking the technique. It is of particular interest to note that none of these artificially injected eggs showed the presence of these acid-fast granules.

As previously mentioned two egg-medium slants were streaked with the sediment obtained by centrifuging, the sediment being suspended in six per cent HCL. These cultures were then incubated at 37°C. in a horizontal position. All tubes showing any growth during the first five days after inoculation, were considered as being contaminated and consequently discarded. Approximately 16 per cent of the cultures became contaminated with molds, after a period of three to five weeks, which necessitated their rejection.

After the cultures had been in the incubator ten weeks, they were examined both microscopically and macroscopically. Two smears were made from each tube, whether they had a characteristic growth or not. If the growth on the slant was characteristic of the M. tuberculosis, and the smears were found to be a

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pure culture of acid-fast organisms, it was considered as positive proof of the presence of tubercle bacilli. If the growth on the slant was sparse, or was not true to type. or the stained smears of the culture were in any way doubtful, the growth was suspended in sterile saline solution. Two cubic centimeters of this suspension was injected into a pullet which had never been exposed to tuberculosis and which reacted negatively to the tuberculin test. Bird No. 4670 was injected intramuscularly and the others intravenously. Four such injections were made, the results of which are shown in Table 3.

Date	1	No.	Test No. 1	Test No. 2	Autopsy
Apr.	12'	5645	_	+++	June 2 - Miliary tuberculosis of liver and spleen; many organisms seen in stained smear from lesions.
	ז ז ז	T T T			July 1 - A guinea pig injected with the culture showed no lesions on autopsy.
Apr.	16'	7052	no test	+++	July 1 - Miliary tuberculosis of liver and spleen.
June	14'	4670' '' ''		++	July 14 - Large localized lesion at point of injection. Small pin-head sized foci in liver. Organisms were observed in stained smears of above lesions.
June	14'	N8 ''	-	+	July 17 - Many small tubercles in liver and spleen. Organisms observed in smears of tubercles of both organs.

Table 3.

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Explanation of Table 3.

In column 1 are dates on which birds were injected.

In column 2 are numbers of birds from whose e_{Sgs} the culture was grown.

In column 3 are the reactions of the birds to the tuberculin tests.

In column 4 are the autopsy findings.

Over the course of three months. 93 egas were examined. Of these 93 eggs, pure cultures were grown from 13. It is thus seen that 13.96 per cent of the eggs laid by these tuberculous hens actually contained the living tubercle bacilli. These eggs were laid by six hens or by 30 per cent of the flock. Many of the remaining 70 per cent went out of production or died from secondary infections too early in the investigation to be of value. Of the four eggs used as checks, the bacilli were recovered from only two. If recovery from eggs positively known to contain many tubercle bacilli was only 50 per cent positive. one can readily see that a great error may be due to the imperfect technique now available for examining eggs for the presence of this organism. In this connection, it might be stated, that in examining with the usual procedure four yolks taken from a naturally infected hen at autopsy, tubercle bacilli were cultured from two of the yolks only. although it seems logical to

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suppose that if they were present in two yolks the other two should be found to contain them.

One striking fact may be pointed out in regard to the eggs from which the pure cultures were isolated. With one exception, all these eggs were laid by birds in whose eggs the acid-fast granules had previously been observed. This exception being bird No. 579, an egg from which was found to contain tubercle bacilli five weeks after an intramuscular inoculation. This bird went out of production almost immediately so further tests were impossible. Opposed to this fact, is the case of bird No. 4668, eggs from which twice showed the presence of acid-fast granules, but repeated examinations of the eggs from this bird failed to demonstrate the <u>M. tuberculosis</u>.

Table No. 4 contains a tabulation of data relative to the birds whose eggs contained either the acid-fast granules or from which the pure culture of <u>M. tuberculosis</u> was isolated. It is of importance here to point out that the bacilli were cultured from the eggs approximately the sixth week after the initial inoculation of the emulsion into the bird.

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No. of bird 579 4590 4668 4670 5645 7052	I Mode of injection Intramuscular Per os Intramuscular Intravenous Intravenous	Reaction tuberculin 4 Weeks	6 t t t t t t t t t t t t t t t t t t t	Time after injection before acid-fast granules lat found 55 days 26 days 44 days	No. eggs from which bacilli isolated One One Four Four One	Time after injection when lst pure culture obtained 46 days 44 days 37 days 39 days
7173	'Intramuscular'	+ +	 +	o' days	Three	SY DRYS

Table 4.

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All birds were autopsied immediately after death, particularly careful examination being made of the organs directly connected with the function of egg production. On the second of May all remaining birds of the flock were killed. This was necessary because the egg production was so low that it made further work with this flock impractical. These birds were autopsied immediately after being killed. Evidence of the tubercle bacilli was sought both macroscopically and microscopically. In Table 5 are recorded the autopsy findings of the seven birds from whose eggs either the acid-fast granules were observed or the pure cultures of the M. tuberculosis were grown. . • •

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Table 5.

No. of bird	Reaction to tuberculin test at six weeks	Died - weeks after inoculation	Autopsy findings
579 1 1 1 1 1 1 1 1 1 1 1 1 1 1	+++ 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	15 weeks (died)	Extensive lesions at the point of inoculation. Many nodular lesions on the liver, spleen, diaphragm, peritoneum, mesentary, the myco- cardium, and the semi- lunar valves of the heart. Lesions in the lungs. At death the ovaries were non- functional, no acid- fast organisms in smears made from them.
4590 ''	+++ " " "	15 weeks (killed)	'Small miliary foci in 'liver and spleen. Ovaries 'functional
4670	++ 1	15 weeks (killed)	Miliary foci in liver. A hemorrhagic cyst-like nodule found in the middle third of the uterus. A smear of the nodule showed the typical <u>M. tuberculosis</u> . Ovaries functional.
5645 " "	++ 1	ll weeks (died)	Miliary tuberculosis of the liver and spleen. Numerous pin-head-sized foci in lung, sections of which show many organisms. Ovaries non-functional, no bacilli found in smears.
7052		ll weeks (died)	Miliary tuberculosis of ' liver and spleen. Large ' caseous foci in lung. Ovary ' non-functional, no acid- ' fast organisms found in ' smears.
7173	++ 1	l2 1/2 weeks (killed)	Small nodular lesions of liver and spleen. A caseous focus in muscle at point of inoculation. Ovaries functional, no acid-fast organisms found in smears.

In noting the data obtained from autopsy of the birds whose eggs were found to contain the M. tuberculosis. it is interesting to point out the case of bird No. 4670. Pure cultures of the strain were grown from four of the eggs of this fowl which on autopsy was found to have a hemorrhagic cyst in the middle third of the uterus in which acid-fast organisms were observed. This is the only bird of the six showing any macroscopic evidence in the reproductive system which would lead one to suspect that the M. tuberculosis might be present in the egg. While the ovaries of four of the six were found on autopsy to be non-functional, there was no macroscopic or microscopic evidence of any tuberculous infection. It was found on the autopsies of all of the 18 birds. two not being autopsied, that the ovaries of 61 per cent of the birds were functional at the time of death. However, 63.6 per cent of the birds whose ovaries were found functional, died early in the experiment from secondary concurrent diseases. The ovaries of the birds that lived 12 months or over were found on autopsy to be functional in 57 per cent of the birds. It may be at this time. stated that although no evidence of infection of the ovaries was observed in any of the birds used in this experiment, unmistakable evidence was obtained on the autopsy of two of the naturally infected hens brought to this clinic from the field. On May 12th a hen infected with miliary tuberculosis was brought in for autopsy. The ovary of the bird was found to

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possess a few small grayish nodules. Stained smears of the material from these nodules showed many acidfast granules, and also many rod-shaped tubercle bacilli in their characteristic arrangement. On June first, a hen showing marked loss in weight was brought in for diagnosis. On autopsy several large tuberculosis nodules were found in the intestine and mesentery. The ovary was non-functional and was seen to possess the small grayish nodules which were observed in the above The stained smears of material taken from them case. were found to contain many of the typical acid-fast tubercle bacilli arranged in their characteristic clusters. No explanation is offered as to why these lesions should be found in the ovaries of the naturally infected birds and not in the ovaries of the birds used for this investigation. The percentage of tuberculous birds which have tubercle lesions in the reproductive organs is exceedingly low. This statement is substantiated by the fact that stained smears made from the ovaries of the artificially infected birds failed to reveal any evidence of the acid-fast bacilli. Additional proof is given to the statement by the fact that stained sections of the ovaries of many of the birds did not show the presence of M. tuberculosis.

On May fourth a second series of birds was started. The eggs from this experiment were to be used primarily for hatching, to determine the transmission of the tubercle bacilli to the chick from the eggs of tuberculous birds, results of which will be published at a later date. However, tests were made for the presence of tubercle bacilli in a few of the eggs which were infertile or which contained the dead embryo.

The second series was composed of 14 tuberculosis free birds, all of which were in fairly good production. The same cocks were used in this experiment as in the first series. Two of the birds were given the intramuscular injection, five the intravenous, and seven were injected by oral route. The inoculum was given in the same manner as in the previous series. The birds were all leg-banded and trap-nests placed in their coop. Two weeks after inoculation the birds all reacted negatively to the tuberculin test. The results of the tuberculin test after four weeks are seen in Table 6.

Mode of injection	No. tested	No. of positive	% Reactors
Intravenous	· 4	2	50
Intramuscular	2	2	100
Per os	' 7 '	l	14.3

Table 6.

All but three of the birds reacted strongly positive to the tuberculin test after six weeks. The three negative reactors all received the oral treatment. In considering the relative speed with which a sensitivity to tuberculin is set up in the birds, it seems from the results of work done in this laboratory and from the result of tests made in these two series, that the birds receiving the intramuscular inoculations are the quickest to give a positive reaction. No explanation can be offered for this at present.

Eggs from this series of birds were taken from the nest, marked, and placed in the incubator. A new set was put in every third day. The chicks which were hatched were all wing-banded and raised for further The eggs which failed to hatch were observation. immediately candled and examined for the presence of the tubercle bacilli. The technique used for examining the infertile eggs was identical with that used for examining the eggs of the first experiment. The eggs containing the dead embryo were washed thoroughly and immersed in Chandler's Colloidal Iodine for three minutes. After drying completely, the eggs were shelled and the fluid around the embryo poured into a sterile test tube. Three smears from the fluid of each egg were made, stained with the regular acid-fast stain, and examined under the oil immersion lens. The remaining fluid was then mixed with about four cubic centimeters

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of six per cent HCl and a culture made on slants of Petroff's and Lubenau's media.

Seventeen of the eggs which failed to hatch were examined. The smears from two of the eggs containing the dead embryo showed typical acid-fast tubercle bacilli. The smears of the other eggs were negative. Unfortunately all but three of the cultures taken from these eggs showed contamination and it was necessary to discard them. Whether the contamination was due to a greater number of organisms resulting from incubation of the egg or to an error in technique I am unable to say. No growth had been observed on the other three tubes after a growing period of four weeks.

If the above snears which were found to contain the acid-fast bacilli can be taken as direct evidence of the presence of the <u>M. tuberculosis</u> in the eggs, the organism was found in 11.7 per cent of the incubated eggs. This figure would undoubtedly have been higher had not so many cultures become contaminated. This statement is justifiable when we remember that in the first series there were no organisms observed in stained smears made directly from the egg, but those that were isolated were done so by culturing. The fact that the bacilli were observed in stained smears made airectly from the egg may be accounted for by the great increase in numbers due to the multiplication during incubation. Because of the unfortunate contamination

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of the cultures made from the eggs of this series, the author does not feel that any positive statement should be made regarding the per cent of presence of the organism in the egg. It is, however, another positive proof of the presence of the <u>M. tuberculosis</u> in the eggs of infected fowl.

Discussion.

There can be no doubt that the tubercle bacilli are present in the eggs of tuberculous fowls. The percentum of this presence is a matter that can not be positively determined until some technique is developed which will render thorough examination possible and practical; and which will utilize more of the egg material. After centrifuging, there is approximately 2.5 cubic centimeters of the solid material thrown Using the present technique, only about 16 down. cubic millimeters of this sediment are utilized in making the determination for the presence of the bacilli. It is obvious that when only about 6.4 per cent of the total residue is examined, the procedure is at best a hit or miss proposition. It is also obvious that if the number of the organisms was not high, it would be relatively hard to obtain positive proof of its presence: and conversely many of the eggs which might contain the bacilli would be considered negative.

The error involved with the present technique is also evidenced by the fact that the recovery was only 50 per cent in the case of eggs known to contain the bacilli. Realizing that the probable error was 50 per cent, as was the error encountered in the recovery of the bacilli from the artificially injected eggs, it is obvious that no positive statement could be made regarding the per cent of eggs from tuberculous fowls

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which actually contained the organism. The work in this paper actually proved the presence of the organism in only 13.96 per cent of the eggs examined, or 76.04 per cent of the eggs were found to be negative. If in this examination an error of 50 per cent was encountered, due to technique, one might be able to estimate the probable presence of the tubercle bacilli in about 52 per cent of the eggs from infected birds. This figure may seem exceedingly high, but it is the author's opinion that it more nearly approaches the incidence which would be obtained by examination with an error proof technique, were it possible and practical to develop such.

It may be that too much stress has been laid on the finding of acid-fast granules in the eggs examined. However, in the writer's opinion, the presence of the granules is in some way related to the presence of <u>M. tuberculosis</u>. It will be remembered that with one exception these granules were present in the eggs of all the birds whose eggs were found to contain the bacilli; also they were only found in one egg from a bird from whose eggs no tubercle bacilli were isolated. At least this subject should offer a field for further study.

A few comments ought to be made regarding the autopsy findings. Tuberculous lesions were found in the organs directly involved in egg production, in

one artificially infected case and in the ovaries of two naturally infected birds. Four of the eggs from hen No. 4670, which possessed a hemorrhagic cyst in the middle third of the uterus from which Lycobacterium tuberculosis was isolated, were found to contain the bacilli. It is common knowledge that the bacilli are found in the blood of infected birds at various times. It is certainly obvious that the bacilli would have ample opportunity to get into the vascular system when we consider that there were lesions found in such organs as the heart and lungs. Would it not be possible for the organism to be transmitted to the egg by this means? It is for this reason that the author does not regard the dearth of lesions found in the reproductive organs, to be a serious argument against the presence of tubercle bacilli in the eggs.

Various workers on this subject have interpreted the results of their investigations in different ways. Nearly all of them state that the incidence of <u>M.</u> <u>tuberculosis</u> in eggs of infected birds is so low that it is unnecessary to consider the matter seriously. It is the opinion of this writer that even if the per cent of the eggs containing the bacilli were only 13.96 per cent it would be great enough to warrant some serious concern.

The present day trend seems to be toward having

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eggs hatched in a hatchery. This may be to some extent responsible for the spread of avian tuberculosis. The work of Fitch and Lubbehusen (13) shows positively that eggs containing the tubercle bacilli, introduced by artificial injection, will not only hatch, but will produce chicks which have tuberculosis. I believe, therefore, it is reasonable to assume that the eggs of naturally infected birds will produce tuberculous chicks. Even though the percentage of infected chicks was very low, we must consider this mode of transmission as an important factor, when we remember that one infected bird in a flock exposes the entire flock to the disease. If a hatchery were to ship out one or two such chicks to a poultryman and these chicks were placed in contact with others of his flock. might not the disease disseminate throughout the flock? Another source of danger from infected eggs is the practise of feeding those which fail to hatch, to chicks, or of throwing them out to swine. There could be no better way of infecting chicks than by feeding them eggs in which the tubercle bacilli have been able to multiply due to the incubation. It would be a step toward the elimination of this disease if all hatcheries would receive eggs only from tuberculin tested stock.

One factor which up to the present time has received very little consideration is the infection of man by the avian strain. In the last few years many workers have reported the finding of human infection

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due to M. tuberculosis avium. The investigations have not stated whether the infected were city or country people. This fact would have been of interest. Many raw eggs are used in egg-nogs, egg-malteds, and other drinks. If eggs from tuberculous birds were used for this purpose, it is conceivable that an intestinal infection might result. The only method of killing the bacilli is by boiling the eggs for five minutes. This procedure is, of course, impossible when the eggs are to be used in the raw state. The only safe source of eggs is from tuberculin tested stock. The tuberculin test is not used as extensively as would be desirable. In many states, however, great advancement has been made in the tuberculin testing of fowls, and it is hoped that this disease may eventually be eradicated. With the elimination would go the dangers derived from eggs containing the bacilli.

Summary.

The presence of M. tuberculosis was demonstrated in 13.96 per cent of the 93 egrs examined. The bacilli were actually cultured on Petroff's. Dorset's or Lubenau's media. Four of these cultures were injected into the inguinal region of tuberculosis free pullets. and on autopsy these birds were found to have tuberculous lesions in various stages of advancement. With one exception, acid-fast granules were observed in smears made from the eggs of all the birds from whose eggs the rod-shaped tubercle bacilli were isolated. The granules were found in one egg of a bird from whose eggs no tubercle bacilli were isolated. The organisms were recovered from only 50 per cent of four artificially infected eggs. Organisms were observed in two smears made from liquid in an egg which had been incubated 22 days, and which contained a dead embryo.

The autopsy revealed lesions of the reproductive system in only one of the artificially infected hens. This was in the middle third of the uterus. Four of the eggs of this bird were found to contain tubercle bacilli. The ovaries of two naturally infected birds brought in from the field, were found to possess several small grayish lesions, stains of which demonstrated typical tubercle bacilli. Many tubercular nodules were found on the organs directly connected with the vascular system of several of the birds autopsied. This latter fact undoubtedly accounts for the transmission of the <u>M. tuberculosis</u> to the eggs. Conclusion.

- I. In this work was demonstrated the presence of <u>M. tuberculosis</u> in 13.96 per cent of 93 eggs. No eggs contained the bacilli until the birds had been infected for at least five weeks.
- There seems to be a high correlation between the presence of acid-fast granules and <u>M. tuberculosis</u> in eggs of diseased birds.
- 3. The incidence of infection of the reproductive organs was found to be very low. One artificially infected and two naturally infected birds were found to have tuberculous lesions in the generative organs.

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