THE
JOURNAL OF
COMPARATIVE PATHOLOGY
AND
THERAPEUTICS.

VOL. XXIII.—No. 4. DECEMBER 31, 1910.

PRICE 2s. 6d.

WHAT IS THE COMMON METHOD OF INFECTION
IN TUBERCULOSIS?

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(Continued from p. 250.)

III.

THE DISTRIBUTION OF THE LESIONS IN EXPERIMENTAL CASES
OF TUBERCULOSIS INDUCED BY INHALATION.

Within recent years it has often been contended by those who uphold the ingestion theory of primary pulmonary tuberculosis not only that that type of disease can be experimentally produced by feeding, but also that it is difficult or impossible to determine primary intrathoracic lesions by causing animals to inhale tubercle bacilli. Calmette and his school have been strenuous supporters of this view of the great resistance which the healthy air passages are able to oppose to direct pulmonary infection with tubercle bacilli, apparently not on account of the results of any inhalation experiments with tubercle bacilli which they have themselves conducted, but because they have persuaded themselves that pulmonary anthracosis cannot be experimentally induced by causing animals to inhale carbon particles suspended in the air. They do not altogether ignore the earlier inhalation experiments with tubercle bacilli, but suggest that any positive results obtained in that way were probably ascribable to the accidental deglutition of bacilli during the course of the experiment. What little ground there is for that suggestion will appear from the experiments which I now propose to quote.

Attention may first be called to two papers bearing on this question which emanated from the Hygienic Institute of the University of Breslau in 1907 and 1908. The authors of these articles (Findel and Reichenbach) carried out researches which were intended to throw further light on the relative importance of infection by inhalation and infection by ingestion in tuberculosis, and the experiments have a peculiar value because they were so designed as to furnish a conclusive answer to the contention of Calmette and others that positive results obtained in animals compelled to inhale tubercle bacilli must be ascribed to accidental deglutition of the bacilli during the experiment. However improbable this suggestion might appear to be, it was impossible to refute it as long as (1) the inhalation experiments were conducted with large unmeasured doses of bacilli, and (2) information was lacking to show the minimum effective dose of bacilli administered by the mouth. Calmette and Guérin not only contended that direct infection of the lungs in inhalation experiments was difficult, but also assumed that comparatively small doses of bacilli were sufficient to ensure infection provided that these were swallowed. In the following experiments account was taken of this assumption by having for each set of animals caused to inhale a measured dose of infective material a control set to which the same dose was administered by the mouth. Immediately this method of experimentation was adopted it became apparent that the minimum effective dose in simple ingestion experiments was immensely greater than the minimum effective dose by inhalation, and as soon as this was established it was easy to show that doses which constantly failed to infect when mixed with the animal's food invariably infected when administered by inhalation in the form of a fine spray. In face of such results the suggestion that infection obtained in inhalation experiments with small doses of material was attributable to bacilli accidentally swallowed absolutely fell to the ground. When, for example, 1 milligramme of bacilli mixed with the food failed to infect, while one-hundredth part of the same material administered as a spray yielded positive results, it became ridiculous to suppose that the small proportion of the sprayed material accidentally swallowed could be held accountable for the animal's infection.

Reichenbach's experiments¹ were carried out with guinea-pigs and goats. In the case of the guinea-pigs the animals had their heads fixed so that they could be exposed to a spray of water with which weighed quantities of culture of human tubercle bacilli were mixed so as to form a fine emulsion. The experiments were conducted in the open air, and during the period of exposure to the spray the animals were perfectly quiet and breathed normally.

The control feeding experiments were carried out with human tubercle bacilli from the same source, and again with carefully weighed quantities of culture thoroughly mixed with a fine carrot pulp. The animals were fasted on the previous day, and they ate the mixture in a short time.

By rubbing up weighed quantities of culture with sterile water Reichenbach prepared a series of emulsions varying in strength. Thus Emulsion I contained .5 milligrammes of bacilli in each cc. of

¹ "Zeitschrift f. Hygiene," Vol. LX., 1908, p. 446.

liquid, and Emulsions II., III., and IV. were made respectively by diluting Emulsion I. ten, a hundred, or a thousand times.

It is, of course, impossible to conduct such spray experiments so as to ensure the inhalation of all the tubercle bacilli in the spray, but in comparing the results of inhalation and ingestion it was assumed that this actually happened, and the comparison was therefore altogether in favour of infection by ingestion. The spray apparatus atomised .02 cm. of fluid in a minute, and in each inhalation experiment the animal was subjected to the spray for ten minutes. Thus, even if none of it were lost the total quantity inhaled was .2 cm., and in each control feeding experiment the quantity of the mixture administered was the same, viz., .2 cm. A calculation showed that 1 milligramme of the culture contained 40,000,000 bacilli.

The result of the experiments was as follows:—

Two guinea-pigs which received .1 milligrammes of culture, or 4,000,000 bacilli, in spray. No. 1, killed after thirty-five days. The lungs were filled with tubercles, mostly about the size of a hemp seed and caseous. The bronchial glands were as large as a hazel nut and caseous. The mesenteric glands were not enlarged. Tubercle bacilli detected in the spleen by microscopic examination. No. 2 died after fifty-seven days. Severe general tuberculosis of the lungs, liver, and spleen. All the lymphatic glands, including the mesenteric, were much swollen and partly caseous.

Two guinea-pigs fed with the same amount. No. 1, killed on the twenty-seventh day, healthy. No. 2, killed on the seventy-second day, also healthy.

Guinea-pigs which received .01 milligrammes, or 400,000 bacilli, in spray. No. 1, killed after fifty-five days. The lungs contained twenty tubercles, of the size of hemp seeds and mostly caseous. Bronchial glands larger than hazel nuts and caseous. Mesenteric glands not swollen. Tubercle bacilli recognised in the spleen by microscopic examination. No. 2, killed after fifty-seven days. Severe general tuberculosis.

Guinea-pigs fed with the same amount of bacilli. No. 1, killed after thirty-seven days, and No. 2, killed after 150 days. Both healthy.

Guinea-pigs which received .001 milligrammes of culture, or 40,000 bacilli, in spray. No. 1, killed after thirty-five days. The lungs contained five tubercles about the size of small peas. Other lesions as in the two previous experiments. No. 2, killed after fifty-seven days. The lungs contained four large and very many small tubercles. Spleen markedly tuberculous. Mesenteric glands swollen.

Guinea-pigs fed with the same amount of material. No. 1, killed after thirty-seven days, and No. 2, killed after 150 days. Both healthy.

Experiment with .0001 milligrammes of culture, or 4000 bacilli, as a dose. In this case both the sprayed and the fed guinea-pigs were found to be healthy when killed.

These facts are sufficiently striking, as they appear to prove the much greater certainty of infection by inhalation as compared with infection by ingestion, and make it absolutely impossible to ascribe the tuberculous lesions which were found in the sprayed guinea-pigs to deglutition of the bacilli contained in the spray.

The difference in the susceptibility of the respiratory and digestive tracts was, however, brought out still more forcibly by the fact that in other experiments guinea-pigs which received respectively 14, million and 1.4 million of bacilli by feeding were found to be healthy when killed after fifty-seven days. The smallest dose which was

found to produce infection by the mouth was 140 million, whereas, as already stated, infection followed in the case of both the guinea-pigs which received only 40,000 bacilli in the form of spray.

A fact previously mentioned must here be emphasised, viz., that, whereas each fed animal consumed the entire dose of bacilli, the sprayed animals could not have inhaled more than a fraction of the same dose.

Reichenbach calculated from his experiments that, even leaving out of account the loss of bacilli from the wind, the smallest infective dose for guinea-pigs by ingestion was 367,500 times greater than the smallest infective dose by inhalation.

Experiments with Goats.—Special importance attaches to Reichenbach's experiments with goats, inasmuch as Calmette laid particular stress on his infection experiments with these animals. The experiments were carried out in much the same way as those above described with guinea-pigs, save that the fed animals sucked the mixture out of a flask. The culture employed in this case was of the bovine type.

The experiments were carried out on the 24th April 1907, and thirty-seven days later a goat which had inhaled 1 milligramme of bacilli was killed. The *post-mortem* examination showed the lungs completely filled with tubercles about the size of hemp seeds. At some places, especially in the anterior lobes, the tubercles had become confluent, and some of them showed distinct central caseation. The bronchial lymphatic glands were much swollen. All the other organs were completely normal.

On the 19th June, or fifty-six days after the beginning of the experiment, two of the sprayed goats and one fed goat were killed. One of the sprayed animals had received 1 milligramme and the other 0.1 milligramme of bacilli, and in both these animals there were pronounced pulmonary lesions. In both cases the lungs were beset with tubercles of the size of a cherry and in great part caseous. In both, the bronchial glands were as large as walnuts, and there were calcified places in them. The mesenteric glands were a little larger than normal, but showed no pathological change. All the other organs were completely normal.

The fed goat killed on the same day had received by the mouth 25 milligrammes of bacilli, and when killed it was found to be healthy with the exception that one of the bronchial glands was as large as a bean and the mesenteric glands were decidedly enlarged, and showed in their outer part a number of calcified points. Distinct tubercles could not be recognised by the naked eye. The microscopic examination of the mesenteric glands revealed the presence of numerous tubercles with caseated and partly calcified centres. At the periphery there were numerous giant cells and some badly-staining tubercle bacilli.

A second fed animal which had been given 5 milligrammes of bacilli by the mouth was killed 104 days after the beginning of the experiment. At the *post-mortem* examination no lesions could be found in any organ, save that the mesenteric glands were enlarged and showed a few calcified points. Microscopic examination revealed marked swelling of the lymph follicles but no other alterations.

It is hardly necessary here to emphasise the fact that, whereas only doubtful or inconsiderable lesions were found in the goats which had swallowed respectively 5 and 25 milligrammes of bacilli, and that in these animals no lesions of any kind were found in the thoracic cavity, severe thoracic lesions were found in the two goats which

received by inhalation respectively the tenth and the hundredth of a milligramme.

The conclusions drawn by Reichenbach from these and other experiments, which space will not permit me to summarise here, were as follows :—

In all animals in which tuberculosis can be set up by feeding infection can be produced more certainly and rapidly, and with much smaller doses, by inhalation. The lesions found in animals subjected to inhalation experiments cannot possibly be ascribed to the accidental swallowing of bacilli during the experiment, or to the penetration of the bacilli into the lymphatics of the throat. In so far as any conclusion regarding the method of infection in man can be drawn from animal experiments, the one which ought to be drawn is precisely the opposite to that drawn by Calmette and his co-workers.

Findel¹ carried out inhalation experiments with a calf and two dogs, which had been tracheotomised some time previously. At the time of the experiments the tracheotomy wound had cicatrised, and the animals inhaled the spray exclusively through the tracheotomy tube.

In the case of the calf the utmost amount of bacilli that the animal could have inhaled during the experiment was 3 milligrammes of a bovine culture. The animal became very ill, with elevated temperature, on the sixteenth day afterwards, and it had to be killed on the twenty-fifth day. The *post-mortem* revealed a miliary tuberculosis of both lungs, the anterior lobes being most affected in each organ. The greyish tubercles varied in size from a millet seed to a small pea. The mediastinal and bronchial glands were markedly swollen, but on section showed no recognisable tubercles. The whole of the abdominal organs and glands were perfectly normal.

In the case of the dog experiments calculation showed that—

No. 1 might have inhaled 465 milligramme or 16,270,000 bacilli.

„ 2 „ „ 28 „ 9,800,000 „

„ 3 „ „ 141 „ 4,935,000 „

Nos. 1 and 2 were killed thirty-three days afterwards, and No. 3 thirty days afterwards.

In No. 1 the *post-mortem* showed scattered grey tuberculous nodules throughout both lungs, but most numerous in the anterior lobes. The bronchial glands were not enlarged. The whole of the abdominal organs and glands were normal in appearance, except that there was venous congestion of the liver and kidneys.

In dog No. 2 the whole lung tissue was almost uniformly beset with small grey tubercles, and the bronchial glands were slightly swollen. The liver showed several flat irregular white spots in its peritoneal covering.

In dog No. 3 the lungs showed lesions similar to those in the preceding case. The bronchial glands were swollen, and showed a few small yellow centres. The spleen, liver, kidneys, and mesenteric glands were completely normal.

Microscopic examination revealed the presence of numerous tubercle bacilli in the lungs and bronchial glands of these animals, but no

¹ "Zeitschrift f. Hygiene," Vol. LVII., p. 104.

tubercles or tubercle bacilli were found in the other lymphatic glands examined.

As a control to these inhalation experiments five dogs were fed with a culture of bovine tubercle bacilli, the dose being as follows:—

Dog No. 4,	13	milligrammes or	455,000,000	tubercle bacilli.
"	5,	69	"	2,415,000,000
"	6,	172	"	6,020,000,000
"	7,	4'48	"	156,000,000
"	8,	13'44	"	470,000,000

In the case of the last two dogs the full dose was given at one meal, but in the other three it was spread over from twenty to thirty-eight days.

The *post-mortem* in each of these cases revealed no distinct tuberculous lesions.

Findel also carried out an extensive series of comparative inhalation and ingestion experiments with guinea-pigs, using in the former an ingenious apparatus invented by himself and Reichenbach, and which enabled one to calculate with a fair measure of probability the actual number of bacilli inhaled by each animal. In these experiments positive results were invariably obtained in the case of animals calculated to have inhaled sixty-two bacilli. A positive result was obtained with two animals which according to the calculation had inhaled only twenty bacilli.

As a control to these inhalation experiments fourteen guinea-pigs were fed with doses varying from 19,100 to 382,000, and in no instance was infection produced. Findel therefore did not reach the minimum infective dose by feeding in these cases, but his experiments indicated that that was at least 19,000 times greater than the minimum infective dose by inhalation.

The foregoing experiments were carried out with adult guinea-pigs, but the author also made a few experiments with guinea-pigs from three to four weeks old, and obtained infection by inhalation in two animals which were calculated to have received respectively twelve and five bacilli.

Space will not permit me to refer at any length to two other papers emanating from the Breslau laboratory, and I must content myself with saying that Alexander's¹ experiments showed the immensely greater susceptibility of rabbits to infection by inhalation than to infection by ingestion, while Heymann² found that it was always possible to demonstrate the presence of tubercle bacilli in the lungs, and sometimes also in the bronchial glands, of guinea-pigs killed from one to twenty-four hours after they had inhaled spray containing tubercle bacilli.

In view of the results of the experiments which have just been summarised, since they appear to prove that in species differing as widely as the guinea-pig, rabbit, goat, and dog a primary pulmonary tuberculosis can with deadly certainty be determined by the inhalation of tubercle bacilli, and, furthermore, that in all these animals the dose of bacilli necessary to ensure infection by ingestion is immensely greater than the minimum effective dose by inhalation, one might without straining probabilities assume it to be a general

¹ "Zeitschrift f. Hygiene," Vol. LX., p. 467.

² *Ibid.*, Vol. LX., p. 490.

law, applicable to other species of susceptible mammals and to man himself, that the inhalation of a given number of tubercle bacilli is more dangerous than the ingestion of the same number. It is prudent, however, to exercise a certain amount of caution before extending results obtained in infection experiments with animals of one species to those of a widely different one, and it is therefore fortunate that there is available direct evidence to prove that in this connection the ox falls into line with the guinea-pig, rabbit, goat, and dog.

Kossel, Weber, and Heuss,¹ in 1905, published an account of a number of experiments, the primary object of which was to compare the effects produced respectively by tubercle bacilli of the bovine and of the human type in bovine animals infected by inhalation. In these experiments weighed quantities of artificial culture were rubbed up with 50 cc. of saline solution, and by means of a specially constructed apparatus the experimental animals were allowed for a period of from seven to twelve minutes to inhale air charged with a fine spray of the liquid in which the tubercle bacilli were suspended.

The condensed particulars of the experiments made with bacilli of the bovine type are as follows :—

Experiment 1. A six-months-old calf inhaled about .5 gramme of culture. Duration of the inhalation about twelve minutes. Killed after 171 days.

The *post-mortem* examination showed caseous and partly calcified tubercles in both retro-pharyngeal, a middle cervical, the left submaxillary, and the left sub-parotideal glands. Both lungs were beset with firm round areas varying in size from submiliary grey translucent tubercles up to firm nodules of the size of a pea or larger. On section numerous caseous lesions were found throughout the whole lung tissue, the larger of these being strongly calcified. The bronchial and mediastinal glands contained completely calcified lesions. No abdominal lesions.

Experiment 2. A five-months-old calf inhaled .05 gramme of bacilli. Duration of the inhalation seven minutes. Killed, when dying, after twenty-six days.

The *post-mortem* showed tuberculous lesions in the retro-pharyngeal glands on both sides, bronchial and mediastinal glands, and lungs (numerous). No abdominal lesions. Guinea-pigs inoculated from the spleen became tuberculous, but those inoculated from the liver and kidneys remained healthy.

Experiment 3. An eight-weeks-old calf inhaled .005 gramme of culture. Duration of the inhalation about eleven minutes. Killed after 174 days.

The *post-mortem* examination showed tuberculous lesions in the left retro-pharyngeal, bronchial, and mediastinal glands, and numerous tubercles in the lungs. Throughout its whole extent the omentum showed a yellowish-brown velvety growth, and a similar growth was present on the mesentery and on the serous covering of the spleen and liver. No visible tubercles in these organs or in the renal or portal lymphatic glands. The majority of the mesenteric glands contained numerous yellowish centrally caseated tubercles about the size of hemp seeds. The tubercles in the mesenteric glands showed no distinct calcification, although that was present in other lymphatic glands and in the lung lesions.

Experiment 4. A three-months-old calf inhaled .005 gramme of culture. Duration of the inhalation ten minutes. Died after sixty-two days.

Post-mortem examination showed tuberculous lesions in the left retro-

¹ "Tuberkulose-Arbeiten aus dem Kaiserlichen Gesundheitsamte," 3 Heft, 1905.

pharyngeal, the mediastinal, and the bronchial glands. Numerous tubercles and areas of caseous pneumonia in the right lung. No abdominal lesions, but two guinea-pigs inoculated with spleen pulp became tuberculous.

Experiment 5. A three-months-old calf inhaled '005 gramme of culture. Duration of inhalation ten minutes. Killed, when very ill, after thirty-seven days.

Post-mortem examination showed tuberculous lesions in the bronchial and mediastinal glands, and very numerous tubercles throughout the whole of the lung tissue. No visible abdominal lesions.

Experiment 6. A four-months-old calf inhaled '001 gramme of culture. Duration of inhalation ten minutes. Killed, when dying, after forty-eight days.

Post-mortem examination showed tuberculous lesions in the bronchial and mediastinal glands and lungs (numerous). No abdominal lesions.

Weber and Titze¹ carried out a valuable set of experiments designed to ascertain the relative susceptibility of calves to infection by inhalation and ingestion with measured quantities of culture of bovine tubercle bacilli.

Having regard to Calmette's views regarding the importance of having the culture material in a finely divided condition, the bacilli were well rubbed up, and in the case of the feeding experiments thoroughly mixed with milk, which the calves drank out of a pail in the natural way. The inhalation experiments were conducted with the apparatus used in the previously described inhalation experiments by Kossel, Weber, and Heuss. The experiments were as follows :—

Experiment 1. A three-weeks-old calf on the 7th December 1907 consumed 10 milligrammes of culture in $\frac{1}{2}$ litre of milk. It did not react when tested with tuberculin on the 17th March 1908. It was killed on the 2nd April 1908, and the *post-mortem* examination showed no tuberculous lesions. Guinea-pigs inoculated from the left bronchial and mesenteric glands were found to be healthy when killed after four months.

Experiment 2. A fourteen-days-old calf was infected at the same time and in the same manner as the preceding animal. It was tested with tuberculin on the 27th March 1908, and did not react. It was killed on the 2nd April 1908, and the only lesion found was a bean-sized caseous nodule in the left precural gland. Bovine tubercle bacilli were cultivated from this. The authors were unable to ascribe this lesion to the feeding experiment, and remark that it is strange that the animal failed to react to tuberculin shortly before it was killed.

Experiment 3. A nine-months-old calf was fed on the 3rd December 1907 with 10 milligrammes of culture in milk. It was tested on the 16th March 1908, and reacted to 40·1 C. It was killed on the 31st March 1908.

The retro-pharyngeal glands on both sides were found to be much enlarged, and contained calcareous foci. The right lung contained a tuberculous nodule of the size of a linseed, which was commencing to caseate in the centre.

Experiment 4. A three-weeks old calf was fed on the 7th December 1907 with 1 milligramme of culture in milk. It failed to react to a tuberculin test on the 27th March 1908, and it was killed on the 1st May 1908.

The *post-mortem* showed no tuberculous lesions. Inoculation of guinea-pigs with pieces of lung had a negative result.

Experiment 5. A nine-months old calf was fed on the 3rd December

¹ "Tuberkulose-Arbeiten aus dem Kaiserlichen Gesundheitsamte," 10 Heft, 1910.

1907 with 1 milligramme of culture in milk. It was tested with tuberculin on the 16th March 1908, and reacted to 41.1 C. It was killed on the 31st March 1908, and the *post-mortem* examination showed no tuberculous lesions. Inoculation of guinea-pigs with material from mesenteric, bronchial, and mediastinal glands and lung had negative results.

Experiment 6. A three-weeks-old calf inhaled on the 14th December 1907 $\frac{1}{100}$ th milligramme of culture. When tested with tuberculin on the 18th March 1908 it reacted to 41.4 C. It was killed on the 9th April 1908.

Caseous nodules were present in the mediastinal and left bronchial glands. One small centrally caseated nodule was present in the left lung under the pleura.

Experiment 7. A nine-months-old calf was fed on the 3rd December 1907 with $\frac{1}{10}$ th milligramme of culture. It failed to react to a tuberculin test on the 16th March 1908, and was found to be healthy when killed on the 31st March 1908.

Experiment 8. A calf which was fed on the 7th December 1907 with $\frac{1}{10}$ th milligramme of culture died on the 25th January 1908 from calf pneumonia and enteritis. No tuberculous lesions were found at the *post-mortem*. Four guinea-pigs each were inoculated from the left bronchial, posterior mediastinal, and right and left retro-pharyngeal glands, and eight guinea-pigs each from the mesenteric glands and the lungs. Of these animals only two became tuberculous, viz., one inoculated from the left retro-pharyngeal gland and one from the posterior mediastinal gland.

Experiment 9. A three-weeks-old calf inhaled $\frac{1}{100}$ th milligramme of culture on the 14th December 1907. When tested with tuberculin on the 18th March 1908 the maximum temperature was 40.7° C. It was killed on the 9th April 1908. Caseous nodules as large as hazel nuts were present in the two mediastinal glands, and a pin-head tubercle was present in the posterior mediastinal gland. A miliary centrally caseated tubercle was present under the pleura of the right lung.

Experiment 10. A three-weeks-old calf was fed on the 7th December 1907 with $\frac{1}{100}$ th milligramme of culture. Tuberculin test on the 27th March 1908 negative. Killed on the 10th April 1908, and found to be free from tuberculous lesions.

Experiment 11. A nine-months-old calf was fed on the 3rd December 1907 with $\frac{1}{100}$ th milligramme of culture in milk. Tuberculin test negative on the 16th March 1908. *Post-mortem* examination on the 31st March 1908 also negative.

Other feeding and inhalation experiments in which smaller quantities than $\frac{1}{100}$ th milligramme were used all had negative results. In summing up these experiments the authors say that they show that for the successful infection of a calf by feeding not less than 10 milligrammes of bovine tubercle bacilli are necessary, whereas infection by inhalation may succeed with a one thousand times smaller dose, viz., with $\frac{1}{100}$ th milligramme. It is pointed out that in reality, however, this comparison is too much in favour of infection by ingestion, since only a very small proportion of the total quantity of culture used in the inhalation experiments was actually inhaled, whereas practically the whole of the culture used in the ingestion experiments was swallowed by the animals. Attention is also called to the fact that four months after infection the animals either showed macroscopic tubercles or were completely healthy, the latter being proved not only by the apparent absence of lesions at the *post-mortem* examination, but also by the negative result of the tuberculin test shortly before slaughter, and in several

cases by the negative result of the inoculation of guinea-pigs with material from the glands and organs. In one case only (Experiment 5) was the tuberculin test positive although the *post-mortem* showed no lesions and the inoculation experiments had negative results.

The last experiment which may be quoted because of its bearing on the point now being considered is one carried out by Svensson. Special interest attaches to it because the conditions under which it was practised approached more closely than usual to what may be called natural circumstances.¹

Fifteen sucking calves, aged from ten to twenty-seven days, and selected from a herd known to be free from tuberculosis, were placed in a special part of a building which also contained eighty cows and some steers. Sixty per cent. of these older animals had reacted to tuberculin.

The intention was to submit the calves to the risk of aerial infection, and at the same time to protect them as far as possible from the risk of infection by the mouth. With that object a space of 6 metres in breadth was left between the calves and the old cattle. During the first month the calves were fed with boiled milk, and after that they received small quantities of dry food, the most painstaking care being taken to ensure the freedom of this food from infective material.

Seven of the calves were introduced on the 14th November, four on the 21st November, three on the 5th December, and one on the 10th December, 1903. One calf died on the 5th February, and a second on the 7th March, 1904 from an intestinal affection, and both were found to be free from tuberculosis. On the 12th and 13th April 1904, or about six months after the beginning of the experiment, the thirteen remaining calves were tested with tuberculin, and five of them reacted. On the 18th April 1904 these five calves were slaughtered, and they showed some small almost miliary tubercles in the mediastinal glands. In addition, two of them had similar lesions in the bronchial glands, and one of these two had also two small tuberculous centres in the lungs.

The eight remaining calves were again tested with tuberculin on the 6th and 7th November 1904, and on this occasion they all reacted. On the 17th November 1904, about one year after they had been placed in the infected building, they were killed. They all showed tuberculosis of the mediastinal and bronchial glands, and one of them also had a pea-sized nodule in the right lung.

If this experiment stood by itself it would be a mistake to attach great importance to it, or to attempt to interpret its results with any great confidence, because it may fairly be objected that when healthy calves are kept under the same roof with tuberculous cows, although separated by a space of 6 metres, aurally transported bacilli may be swallowed as well as inhaled by the former. But when the experiment is read in conjunction with those previously described it appears quite reasonable to hold that the intrathoracic lesions found in the infected calves were caused by inhalation and not by the ingestion of bacilli.

¹ Original report not accessible. Reference in "Centralblatt. f. Bakter.," Ref., Vol. XLII, p. 29.

IV.

PULMONARY ANTHRACOSIS.

It is a familiar fact that both in men and in animals which have for any considerable period lived in a smoky atmosphere the lung tissue and the bronchial glands are often visibly pigmented with carbon particles.

Prior to the year 1905 it was generally accepted that the pigment was composed of soot particles which had been suspended in the atmosphere and had reached the lung tissue direct with the inhaled air. The pigmentation of the glands was ascribed to the transport of the same soot particles from the lung tissue by way of the lymphatic vessels.

Very little reflection will show that as part of the case put forward by Calmette and others in favour of the intestinal origin of pulmonary tuberculosis it was necessary to show that this view of the genesis of anthracosis of the lungs and bronchial glands was erroneous. At Calmette's suggestion the question was experimentally investigated by Vansteenberghe and Grysez,¹ and as a result they maintained that the easiest method of producing a typical pulmonary anthracosis in adult guinea-pigs consisted in mixing Chinese ink or carbon powder with their food. They declared that when the animals were killed twenty-four hours after such a repast the pulmonary parenchyma showed disseminated black spots, especially in the upper lobes and along the edges of the lower lobes. In these cases the mesenteric glands were normal, but those of the mediastinum were swollen and black.

They found that when young guinea-pigs were used for experiment the results were completely different, inasmuch as the lungs showed no pigment, while the mesenteric glands were absolutely infiltrated with carbon particles.

In support of their contention that pulmonary anthracosis is due to intestinal absorption of the pigment, they also reported that when the œsophagus had previously been ligatured pigmentation of the lungs could not be produced by causing the animals to inhale a smoky atmosphere except when the experiment was very prolonged.

As was to be expected, these statements attracted much attention, and quite a large number of experiments were soon afterwards carried out by various observers in order to determine whether the new view put forward regarding the intestinal origin of pulmonary anthracosis was correct or not. Space will not permit me, nor does it appear necessary, to describe all these later investigations regarding this question. The following must suffice.

Beitzke² repeated the experiments of Vansteenberghe and Grysez and obtained diametrically opposite results. He upheld the older view as to the respiratory origin of pulmonary anthracosis, and contended that when the condition was found in animals experimentally fed with carbon mixtures the result was due to aspiration of the mixture during feeding. When precautions were taken to pre-

¹ "Annales de l'Institut Pasteur," 1905, p. 787.

² Virchow's "Archiv," Vol. CLXXXVII., p. 183.

vent this accident pulmonary anthracosis was never found in the experimental animals.

Kuss and Lobstein¹ found that it was possible to produce pigmentation of the lungs and bronchial glands by causing animals to inhale carbon for twenty minutes morning and evening for three weeks. In their experiments it was found that when the same quantity of Chinese ink was administered to some animals by inhalation, and to others by ingestion, only the former showed anthracosis. It is true that they found that when massive doses of Chinese ink were introduced directly into the stomach or into the duodenum one might find in the animals killed, from twelve to thirty hours afterwards, some small sub-pleural points of pigmentation, but in order to produce a condition comparable with natural anthracosis it was necessary to inundate the intestine with Chinese ink.

They therefore concluded that the ordinarily observed anthracosis is ascribable to inhalation and not to deglutition.

Nieuwenhuyse,² as the result of experiments carried out on thirty-five guinea-pigs, concluded that pulmonary anthracosis is not of intestinal origin.

Arloing and Forgeot³ carried out experiments bearing on this question with guinea-pigs, rabbits, goats, and dogs (young and adult), which were given by ingestion Chinese ink, charcoal, or pigment from a melanotic tumour in a horse. The animals were killed from six to forty hours afterwards. In no case did they find any anthracosis of the intestinal wall or mesenteric glands. They concluded that if such solid particles do ever transverse the intestine it can only be in exceptional cases and not under physiological conditions.

Experiments by Whitla and Symmers.

Sir William Whitla, who in 1908 delivered the Cavendish Lecture before the West London Medico-Chirurgical Society, chose as the subject of his address *The Etiology of Pulmonary Tuberculosis*,⁴ and sought to show that recent researches had completely overthrown the previously accepted view that inhalation is the common method of infection in man. In the course of his address he outlined the researches of Calmette and Guérin, and Vansteenberghe and Grysez, regarding tuberculosis and pulmonary anthracosis, and adopted the conclusions of these authors with enthusiasm. He characterised the experiments of Calmette and Guérin as epoch-marking, declared that they had shifted the question of the portal of entrance of the tubercle bacillus from the site of the pulmonary aveoli to that of the intestinal epithelium, and prophesied that probably at no distant date it would be accepted that "in the immense majority of cases pulmonary tuberculosis is not contracted by inhalation, but, as taught by Von Behring, the germs enter through the intestinal tract." In support of this view he described certain experiments which he had carried out in conjunction with Professor Symmers regarding the production of pulmonary anthracosis. The results of these experiments were in complete accord with those obtained by Vansteenberghe and Grysez,

¹ "C. R. Acad. des Sciences," Vol. CXLIV., p. 162, and "C. R. Soc. Biologie," Vol. LXII., p. 139.

² Reference in *Bulletin de l'Institut Pasteur*, 1907, p. 472.

³ "C. R. Acad. des Sciences," Vol. CXLIV., p. 786.

⁴ "British Medical Journal," 1908, p. 61.

in that more or less marked pigmentation of the lungs was found in adult guinea-pigs into whose stomachs large quantities of Chinese ink had been introduced even as short a time as four hours before they were killed. In some cases the animals' lungs exhibited "an almost ebony-like blackness."

Before attempting to explain the results of these experiments it may be observed that even if they admitted of no other explanation than the one put forward by Sir William Whitla, viz., that the carbon particles had reached the lung tissue from the intestine by way of the thoracic duct, they could not be accepted as proof that the tubercle bacilli which cause pulmonary tuberculosis reach the lung by the same route, in view of the results obtained by Findel, Reichenbach, Alexander, Heymann, and others, in the experiments to which I have already referred.

It may, however, with some confidence be suggested that Whitla and Symmers, as well as Vansteenberghe and Grysez, have misinterpreted their results, through failing to take account of certain possibilities of error involved in their method of experimentation.

In the first place, it must be noted that they appear to have neglected the possibility that the anthracosis which they found in their experimental animals was spontaneous. The force of this suggestion will appeal to anyone who has paid attention to the frequency with which pulmonary anthracosis is found in healthy adult guinea-pigs. It is true that "almost ebony-like blackness" of the lungs from spontaneous anthracosis is never found in guinea-pigs, and if Whitla and Symmers found such a condition in guinea-pigs which simply ingested food mixed with Chinese ink I, for one, must declare myself unable to offer any certain explanation of the occurrence. At any rate, the authors' explanation cannot be accepted as reasonable, for they admit that in these cases the abdominal lymphatic glands, through which the pigment was supposed to pass, were of "ivory-like whiteness and free from any obvious impregnation with carbon particles"!

But in Whitla and Symmers' experiments some of the animals had the Chinese ink, rubbed up with olive oil and water, introduced into their stomachs by means of a soft india-rubber catheter passed through the mouth, pharynx, and œsophagus. This must be regarded as a dangerous procedure in experiments in which it was necessary to exclude the possibility of direct admission of the pigment-containing liquid into the air passages, and it is therefore quite possible that aspiration of the liquid was the explanation of the ebony-black lungs found in some of the animals.

Probably, however, the main source of error in these experiments was that the authors did not attach sufficient importance to the frequency of spontaneous anthracosis in adult guinea-pigs. This suggestion finds striking support in the fact that the authors were unable to find pigmentation of the lungs when they selected young guinea-pigs. "In ordinary feeding experiments when the carbon was mixed with the food of these young animals we found usually that neither the glands nor the lungs became visibly infiltrated with carbon particles, the intestinal contents alone being black."

It is unfortunate that Sir William Whitla, in asking the medical profession in this country to abandon the view that inhalation is the

common cause of pulmonary tuberculosis, omitted any reference to the numerous experiments which had already been published at the date of his lecture with results diametrically opposite to those claimed to have been obtained by Calmette and Guérin, and Vansteenberghe and Grysez.

A review of all the experiments which have been carried out in order to determine the mechanism of pulmonary anthracosis will, I think, compel any impartial person to conclude that Vansteenberghe and Grysez's conclusions were founded on error. The older view, viz., that the carbon particles are carried directly into the lung tissue with the inhaled air, has been thoroughly vindicated, and the contention that a degree of pigmentation of the lung tissue and bronchial glands comparable with what is commonly encountered in men and animals can be produced experimentally by feeding with any amount of carbon or Chinese ink must be considered disproved by the weight of experimental evidence.

In reality, however, any discussion of the route by which the soot particles reach the lungs in natural cases of anthracosis has become quite superfluous when one is considering whether primary pulmonary tuberculosis is caused by the inhalation or by the ingestion of tubercle bacilli. It is now absolutely vain to cite the experiments of Vansteenberghe and Grysez, since, as has already been shown, the possibility of producing a direct infection of the lung with tubercle bacilli by inhalation has been absolutely proved.

V.

CONCLUSIONS.

Conclusions which appear to be justified after a review of all the available evidence are the following :—

(1) The inhalation of tubercle bacilli suspended in the atmosphere is a very certain method of infection in susceptible animals even when small doses of bacilli are employed.

(2) Experimental infection with tubercle bacilli by way of the alimentary canal is comparatively difficult to realise even in highly susceptible animals, and success is certain only when very large doses of bacilli are administered.

(3) With few exceptions, in animals experimentally infected with tuberculosis by way of the intestine the primary lesions are intra-abdominal, and the intrathoracic lesions when present are secondary.

(4) Inhalation is probably the commonest natural method of infection in those species (man and cattle) in which the primary lesions of tuberculosis are usually intrathoracic.

(5) Naturally-contracted cases of tuberculosis in man and other mammals can be ascribed to infection by ingestion only when the lesions revealed at the *post-mortem* examination are confined to the abdomen, or when the existing abdominal lesions are recognisably older than those present elsewhere in the body.

In formulating these conclusions I have endeavoured to evade the reproach to which Calmette and his supporters have laid themselves open, viz., that of drawing far-reaching inferences from a small number of experiments, and assuming that the results obtained in

animals under the conditions realisable in experiments may be immediately applied to explain the method of infection in cases of natural tuberculosis in man. That is why the word "probably" has been introduced into the fourth of the conclusions. The whole of the experimental evidence on which the theory of the intestinal origin of pulmonary tuberculosis in man was built up has been swept away, and valuable new support has been provided for the older inhalation theory; but one ought to avoid the mistake of denying any importance to infection by ingestion either in man or in cattle, or of asserting that tubercle bacilli which enter the body by way of the alimentary canal are never the cause of tuberculosis with lesions apparently primary in the lungs.

SOME POINTS IN THE EPIZOOTIOLOGY OF SHEEP SCAB IN RELATION TO ERADICATION.

By S. STOCKMAN, Chief Veterinary Officer, Board of Agriculture.¹

NOTWITHSTANDING the fact that sheep scab is one of the oldest diseases known, its epizootiology still presents unsolved problems which are of considerable importance in State medicine. The policy followed during the last three years in dealing with sheep scab in Great Britain has been of a tentative character, and the plan of campaign, which has been to reduce gradually the prevalence of the disease before resorting to more heroic measures for its final eradication, has been largely dictated by the exigencies of the business of sheep farmers. In the Reports of the Chief Veterinary Officer, Board of Agriculture, for 1906, 1907, and 1908, references were made to what appeared to be defects in the tentative methods adopted, and a continued study of the circumstances connected with the various outbreaks has since tended to confirm opinions which formerly might have been considered to have been based upon insufficient evidence from the field.

The Report of 1906 pointed out that "one must not hastily conclude that the recognised and confirmed outbreaks represent the sum total of sheep scab in the country, as indications are not wanting that quite a number of cases in an early or comparatively quiescent stage are not diagnosed until a later date, when more marked clinical symptoms supervene. These remarks especially apply to flocks of sheep which have been dipped under one or other of the compulsory dipping Orders, and at the time of dipping were not noticed to be amiss. In some instances, however, after an interval of two or three months' time, these sheep, although they have not been in contact with others, have nevertheless developed clinical and unmistakable appearances of sheep scab, and an outbreak has been declared. That such cases are not altogether exceptional there is good reason to believe, and a method of combating them is deserving of careful consideration."

It was stated in the Report of 1907 that "in not a few instances only one animal out of a large flock was returned as affected after

¹ From the Annual Reports, 1906-7-8-9.