A COMPARATIVE STUDY OF BOVINE TUBERCLE BACILLI AND OF HUMAN BACILLI FROM SPUTUM

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FROM
THE JOURNAL OF EXPERIMENTAL MEDICINE
VOL. III, NOS. 4 AND 5, 1898

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The absolute identity of tubercle bacilli infecting mammalia has been so generally assumed and the assumption used as a basis for the enactment of sanitary measures having for their object the prevention of any transmission of tubercle bacilli from animals to man, that any one who would attempt to question this identity must be prepared to meet considerable scepticism. Taking a broad biological position, we have every reason to examine into the assumed identity of the bovine and the human bacillus, because both the human and the bovine species are victims of a tuberculosis, presumably transmitted in most cases from one individual to another of the same species, and because the adaptation of a highly parasitic organism to one of these species for centuries may possibly deprive it of much of its power to multiply in the other. Or the reverse may be true. The adaptation of the bovine bacillus to a larger, more vigorous organization may thereby render it more dangerous to man. Assumptions of this sort stimulate inquiry but do not furnish us with positive information. This can be gained only by most tedious investigation.

In 1896 I presented to the Association of American Physicians* a comparative study of a bovine bacillus and a presumably human bacillus which had passed from a tuberculous subject to an animal pet (*Nasua narica*, 'Coati'). The differences which appeared during this work were so clear, both as regards morphological and biological characters, that a further study was indicated. Imbued with the idea that in the minor, though constant, differences of closely related or apparently identical pathogenic bacteria many unexplained phenomena may

* *Trans. Assoc. Amer. Physicians*, xi (1896), 75.

have their source, and that a comparative study of tubercle bacilli under as nearly the same conditions as can be maintained may lead to results of value to human pathology, I took up the subject anew. A consultation of the literature treating of the character of tubercle bacilli had impressed me with the carelessness shown by most writers subsequent to Koch, concerning the source of the cultures used by them. Papers written on methods of cultivating the presumably human bacillus were really devoted to the avian bacillus. Fischel,* though drawing far-reaching conclusions on the relation of human to avian bacilli, makes no statement concerning his cultures save that they were "mammalian."

There being, therefore, no information to be gathered from former publications, the task that presented itself was to study a number of human and bovine tubercle bacilli, and, if possible, tubercle bacilli from other mammalia, side by side, in order to determine whether constant differences, of whatever nature, could be demonstrated. Such differential characters might then be applied in studying different forms of human tuberculosis, especially those cases which are supposed to have been derived from animals, more particularly from cow's milk.

The delay inseparable from the study of this disease has not permitted me to examine more than a limited number of cultures. Including the two described in 1896, 7 sputum and 6 bovine cultures have been isolated and studied; also one animal culture presumably derived from sputum, one culture each from the horse, the cat and the pig.

In Table I are given brief accounts of the subjects from which they were obtained. For the notes on the cases of phthisis I am indebted to a number of physicians who have very generously given their time in tracing the subsequent history.

* *Fortschr. d. Med.*, x (1892), 908. See also my former article for further references.

TABLE I.

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
Sputum I	July 20, 1896	Peabody, Mass.	Sputum discharged, June 24, 1896, by a young Irish woman. Apex and upper lobe of left lung involved. Slight fever. Sputum contains a considerable number of bacilli. Recovered and worked during summer. Good health in the fall of 1896, with gain in weight. Feb., 1898, in good health, works daily. No cough, expectoration or other symptoms of illness. Over the formerly involved region "the respiratory murmur much diminished down to 3rd rib. Increased vocal resonance, increased fremitus and dullness on percussion." Patient has moved from place to place.
Sputum II	Nov. 21, 1896	New Bedford, Mass.	Sputum containing many tubercle bacilli, discharged Oct., 1896, by a young man confined in prison about one year. Earliest appearance of symptoms not accurately known. Slight cough noticed in Aug., 1896, but no definite physical signs at that time. Grew steadily worse, so that in November he was in bed, weak and emaciated and also suffering with diarrhœa. He died in March, 1897.
Sputum III	Feb. 15, 1897	Norwood, Mass.	Sputum discharged, Jan. 12, 1897, by a man aged 45 years. Duration of disease about six months. Laryngeal tuberculosis chiefly. Difficulty in swallowing and huskiness of voice, which has been gradually increasing. Died Feb., 1897. The physician in charge had drawn off about a litre of serum from thorax 4 or more years ago. Well thereafter until fall of 1896. Apex of lungs slightly involved towards end of disease. Patient has traveled much in this country.
Sputum IV	Feb. 16, 1897	Melrose, Mass.	Sputum containing but relatively few bacilli, discharged Jan. 7, 1897, by a female servant, 22 years old. Physician in charge saw her first Dec., 1896. She complained of hoarseness and cough which began about 6 months ago. Signs of anæmia and cessation of menstrual flow. Placed in hospital, where she remained 8 weeks. At end of April health quite re-established. A subsequent report mentions her visit to Canada after her recovery and her return to Massa-

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
			chusetts. She is now (Jan., 1898) at service in another town. She has a troublesome cough and is under the care of a physician.
Sputum V	July 7, 1897	New Hampshire.	Sputum discharged, June, 1897, by a man 25 years old. Had been troubled with a cough for about 2 years, and apices reported involved. Patient feels well in the country, but cannot carry on his trade (printer) in the city. In Jan., 1898, he was reported to be much improved in every way.
Sputum VI	July 14, 1897	Winthrop, Mass.	Sputum discharged, June 16, 1897, by a woman 20 years old. Tubercle bacilli numerous. History not obtainable. Patient died about 6 months later.
Sputum VII	Jan. 18, 1898	Haverhill, Mass.	Sputum containing many bacilli, discharged by an Italian laborer, Dec. 15, 1897. Age of patient 27 years. Returned to Italy in Jan., 1898.
Nasua narica (Coati) human sputum (?)	July 26, 1894	Washington, D. C.	Autopsy, May 12, 1894, revealed primary intestinal infection, as mesenteric glands were farthest advanced in the disease. Omentum studded with tubercles. Extensive embolic tuberculosis of lungs. Household pet of tuberculous man. At Washington Zoological Park, where animal was kept after death of owner, other animals of same species remained well.
Bovine I	Dec. 1, 1894	Virginia.	Bull with advanced generalized disease. Great enlargement of lymph glands of head and thorax. Tuberculosis of the bones. Periodic tympanites due to enlarged mediastinal glands. Killed.
Bovine II	Nov. 28, 1896	Lawrence, Mass.	Cow having multiple necrotic foci in large caudal mediastinal gland. Foci dry cheesy; process evidently old. In one lobe of lung a focus 1½ inches in diameter with centre softened. In udder small nodules containing actinomyces. Killed.
Bovine III	April 19, 1897	Carlisle (?), Mass.	Cow killed, March 19, 1897, at Brighton (Boston) abattoir. Quite extensive disease of lungs and liver.

TABLE I.—*Continued.*

Designation of culture.	Beginning of artificial cultivation.	Source of culture.	History of Case.
Bovine IV	Apr. 23, 1897	Billerica, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease restricted to one of dorsal mediastinal glands. The gland is moderately enlarged and contains a number of well-defined tubercles, 3 to 4 mm. in diameter, dry cheesy throughout, and easily peeled out of gland tissue. No surrounding infiltration. Evidently a mild stationary case.
Bovine V	Apr. 23, 1897	Carlisle, Mass.	Cow killed, March 19, 1897, at Brighton abattoir. Disease has produced a focus in the lungs, and involves all dorsal mediastinal glands, which are considerably enlarged. One of the portal glands is enlarged, and permeated with a network of necrotic lines.
Bovine VI	Dec. 24, 1897	Newton, Mass.	Caseous matter from a focus in the lungs of a cow 2 to 3 years old. The focus several inches in diameter, probably not more than 3 to 3½ months old, since the cow gave no tuberculin reaction 4 months ago. Dorsal mediastinal gland involved.
Swine I	May 8, 1896	Southboro, Mass.	Lungs and one gland (presumably from mesentery) received at laboratory, March 18, 1896. The herd lived under a cow stable, and the infection was probably bovine in origin. Lungs studded with firm, centrally cheesy or calcified nodules from 1 to 4 mm. in diameter. The lymph gland contained many foci with cheesy centres.
Cat I	Mar. 9, 1897		Case reported by L. Frothingham, V. M. D., in the Journal of the Boston Society of Medical Sciences, March, 1897. Tuberculosis of a mesenteric gland with miliary tuberculosis of lungs, liver and spleen. Tubercle bacilli in capillaries of lungs and liver. Guinea-pig inoculated by Dr. Frothingham, and kindly transferred to me.
Horse I	Feb. 20, 1897		Lungs of a horse from Brighton Rendering Establishment. Guinea-pig inoculated from tubercles by Dr. L. Frothingham. History of animal not known.

It will be noticed that these cultures come from a variety of cases, both mild and severe. Of the patients yielding the sputum bacilli, three are dead, two still sick, one has apparently recovered, and one, a recent case, is now not accessible. Of the bovine cases two were slight, two advanced and two of moderate intensity. Of the other mammals nothing very definite is known.

The bacilli were isolated in all cases by inoculating guinea-pigs with tuberculous tissue or sputum containing bacilli. With sputum the injection of a suspension in bouillon into the abdominal cavity is the most satisfactory. Subcutaneous inoculation may induce a very chronic disease with older (500-gramme) guinea-pigs, which is not so likely to lead to successful cultures because of the scarcity of bacilli in the tuberculosis foci. In nearly all cases the guinea-pigs were chloroformed after three or four weeks, to prevent secondary infections occurring in the final stages of the disease.

Inasmuch as a more general study of tubercle bacilli is desirable, I shall go somewhat into detail concerning the methods I have employed in making cultures. Having had practically nothing but failures until I adopted the procedure to be described, I can recommend it without reserve. There is nothing new about it, excepting in the combination of details. Of 18 attempts to isolate the bacillus, 17 were successful. The one failure was probably due to the slow progress of the inoculation disease in the guinea-pig.

Throughout the work dog's serum was used. The dog was bled under chloroform and the blood drawn from a femoral artery, under aseptic conditions, through sterile tubes directly into sterile flasks. The serum was drawn from the clot with sterile pipettes and either distributed at once into tubes or else stored with 0.25 to 0.3 per cent chloroform added. Discontinuous sterilization was rendered unnecessary. The temperature required to produce a sufficiently firm and yet not too hard and dry serum is for the dog 75° to 76° C. For horse serum it is from 4° to 5° lower. The serum was set in a thermostat into which a large dish of water was always placed to forestall any abstraction of moisture from the serum. About three hours suffice for the coagulation. When serum containing chloroform is to be coagulated, I am in the habit of placing the tubes for an hour or longer in a water-bath at 55°-60° C., or under the receiver of an air-pump, to drive off the antiseptic. This procedure

dispenses with all sterilization excepting that going on during the coagulation of the serum. It prevents the gradual formation of membranes of salts, which, remaining on the surface during coagulation, form a film unsuited for bacteria. Tubes of coagulated serum should be kept in a cold closed space where the opportunities for evaporation are slight. They should always be kept inclined.

The ordinary cotton-plugged test tubes I do not use, because of the rapid drying out permitted by them, as well as the opportunities for infection with fungi. Instead, a tube is used which has a ground glass cap fitted over it. This cap contracts into a narrow tube plugged with glass wool. This plug is not disturbed. The tube is cleaned, filled and inoculated by removing the cap. With sufficient opportunity for the interchange of air very little evaporation takes place, and contamination of the culture is a very rare occurrence. In inoculating these tubes, bits of tissue, which include tuberculous foci, especially the most recent, are torn from the organs and transferred to the serum. Very little crushing, if any, is desirable or necessary. I think many failures are due to the often futile attempts to break up firm tubercles. Nor should the bits of tissue be rubbed into the surface, as is sometimes recommended. After a stay of several weeks in the thermostat I usually remove the tubes and stir about the bits of tissue. This frequently is the occasion for a prompt appearance of growth within a week, as it seems to put certain still microscopic colonies in or around the tissue into better condition for further development. The thermostat should be fairly constant, as urged by Koch in his classic monograph, but I look upon moisture as of more importance. If possible, a thermostat should be used which is opened only occasionally. Into this a large dish of water is placed, which keeps the space saturated. Ventilation should be restricted to a minimum. As a consequence, moulds grow luxuriantly and even the gummed labels must be replaced by pieces of stiff manila paper fastened to the tube with a rubber band. By keeping the tubes inclined, no undue amount of condensation water can collect in the bottom, and the upper portion of the serum remains moist. The only precaution to be applied to prevent infection with moulds is to thoroughly flame the joint between tube and cap as well as the plugged end, before opening the tube. When test tubes are employed it is well to dip the lower end of the plug into sterile molten paraffine and to cover the tube with a sterilized paper cap. The white bottle caps of the druggists are very serviceable.

In pursuing this method of obtaining cultures, the question arises: May not attenuated or more saprophytic forms exist in the sputum

besides those more pathogenic, and may not the former get into our cultures rather than the latter? This question cannot be answered with entire satisfaction. If we assume, for the sake of illustration, that the original disease was due to perhaps one inhaled bacillus, the progeny of this bacillus may be reinforced by others inhaled which have more saprophytic and less pathogenic tendencies, and which multiply in the cavities and in the sputum to the partial suppression of the original form. If we make cultures directly from sputum this hypothetical danger could not be evaded. The inoculation of the guinea-pig and the three or more weeks elapsing before cultures are made is to a certain degree a safeguard, as it gives the more pathogenic form an opportunity to assert itself by prompt multiplication. I am not inclined to look upon this danger of substitution as a real one. At the same time, it may be best always to choose those successful cultures from the guinea-pig which have come from metastatic rather than local (peritoneal) tubercles. In my work I obtained in most cases cultures simultaneously from 3 or 4 organs, and any differences in these original cultures were not manifest. Only one was used in the comparative studies. Of 7 sputum cultures, 3 were derived from the omentum and 4 from the spleen. Of one the source is not noted. For the bovine cultures no such doubts can be raised, because the foci are closed and not accessible to more saprophytic types.

In the study of the various cultures the conditions were maintained as nearly uniform as possible by using tubes of serum prepared at the same time, and keeping the inoculated cultures in the thermostat in the same trays. For the microscopic examination a little of the surface growth was rubbed between two cover glasses, allowed to dry in the air, and then the whole series to be compared were fixed all together in the hot-air sterilizer at 120° C. for 20 to 30 minutes. For staining, carbol-fuchsin was used cold, and the decolorization effected with a 10 per cent aqueous solution of sulphuric acid acting for from 15 to 30 seconds. Cover-slips were also decolorized with ½ per cent acetic acid, some simply washed in water, others were stained according to Gram-Weigert. In all cases the members of the series studied were treated alike and at the same time, thus eliminating minor, often unavoidable, variations. In all cases the characters to be described refer to dog-serum cultures. These were renewed every 3 to 6 weeks, as many at a time as was possible. The regularity of re-inoculations was now and then interrupted by a beginning infection of the tube with moulds, or a too feeble growth, necessitating

earlier transfer to fresh tubes. As a rule the cultures were kept continuously in the thermostat until the next transfer was made.*

The tendency of most species of bacteria to vary slightly from tube to tube, either in size, form, or staining capacity, is also characteristic of tubercle bacilli, so that repeated examination of series of cultures is necessary to obtain what might be called a composite photograph of their most permanent characters or peculiarities. The statements made below as to the morphology of the bacilli under observation are based on the repeated examination of hundreds of slides of which the conditions of growth of the bacilli, the coagulating point of the serum, and the staining manipulations had been carefully noted. The cultivation of tubercle bacilli requires, even under the best circumstances, constant personal attention, and those not prepared to give it had better not attempt it seriously. Success is obtainable along lines other than those suggested by me. Koch succeeded by using beef's serum in ordinary plugged test tubes. He is, however, the only investigator, I believe, who has studied series of cultures of tubercle bacilli.

MORPHOLOGICAL AND BIOLOGICAL CHARACTERS.

With one exception to be noted below (Sputum I), the human bacilli grew from the start much more vigorously than the bovine bacilli. With several the rapidity of growth was surprising. After two weeks these cultures appear as a whitish surface layer of a pearly lustre, of varying thickness. The bovine cultures show merely discrete colonies, or a thin, uniform layer having the appearance of ground glass.† This difference in the vigor of growth has, in general, maintained itself, with the slowly increasing tendency of all cultures to multiply more rapidly.

The size of bovine bacilli in the various cultures was quite constant. They were all quite short, usually about 1 to 1.5 μ long, more rarely 1

* It is always desirable as a precautionary measure to retain 3 or 4 older transfers of any culture in a cool, dark place, so that it may not be lost through some accident or contamination. Now and then culture-media will prove unsatisfactory. In such cases, the earlier tubes must again be resorted to. I have noticed that upon all the serum tubes from one dog, growth was feeble. The liver and the kidneys of this animal showed considerable fatty changes, although the animal had not been ill. Possibly overfeeding may be the cause of the difficulty.

† In some tubes a peculiar coppery discoloration may extend over the entire surface growth. Cultures from these are fertile, however. There is nothing in the microscopic appearance of the bacilli to explain this change. It is not an uncommon feature of cultures of feeble vitality, or of unsatisfactory culture-media.

to 2 μ. These measurements do not tend to change appreciably with prolonged cultivation. The bacilli are straight, not very regular in outline. Some are broader at one end than at the other, some broader in the middle than at either end, *i. e.* spindle-shaped. Some may be so short as to resemble oval cocci. With the human bacilli the form was not so constant. The earliest cultures of Sput. II, IV, V and VI contained forms from 1 to 2 μ long, hence closely approximating the bovine forms. Others (Sputum III) may be longer from the start. In all, however, there is a tendency, not noticed among bovine cultures, to grow longer under artificial cultivation. Perhaps the greatest contrast in form, which has remained so for three years, is illustrated by the bovine and the Nasua (human) culture described in 1896. In the latter the bacilli are from 2 to 3 times the length of the former.

In the earliest cultures, therefore, morphological differences are not necessarily characteristic, and cannot aid us in attempts at determining the origin of cultures. Cultures containing only small forms have been noted by Metchnikoff and Straus. The latter states that even the avian variety may assume very small dimensions under certain circumstances.

When the serum to be used for cultures is coagulated at a temperature of 71° to 72° C., instead of 75° to 76° C., certain modifications are manifest. All bacilli which have developed on a serum having a firm surface, and which is obtained at the higher temperature, absorb the dye promptly and become brilliantly stained. When a softer (71° C.) serum is employed, whose surface is easily depressed, torn and broken by the platinum needle, the culture, which may have become either more or less vigorous than before, now contains a large number (from 50 to 90 per cent) of bacilli, which stain quite feebly. Uniformly dispersed among them are deeply stained forms. This feeble stain is noticed when bacilli are not treated with decolorizing agents at all, and the appearance of the cover-slip preparation is not materially altered by the acid.*

While both human and bovine bacilli are affected by the softer serum, the former show a much greater degree of change. The bacilli are very feebly stained and show outlines often very indistinct. Further than

* Since the completion of the manuscript further studies of this phenomenon have convinced me that at least some of the feeble staining is due to the formation of a capsular substance, which interferes with the penetration of the dye. This substance, which is produced in greatest abundance by the human cultures, is not infrequently detected as an irregular, unstained envelope around a single bacillus or a group. It is not stained by the procedure recommended by Prof. Welch for staining capsules.

this, the human bacilli, on the softer serum, tend to become longer and slightly curved. In some cultures this change is very pronounced, in others less so. With the bovine forms there is no elongation, although the bacilli may be less brilliantly stained than on firm serum. Of seven human cultures studied, all have exhibited this tendency to become slender, while none of the bovine bacilli have been seen to change their size to any appreciable degree. Some of the bovine cultures from softer serum did not even show any feebly stained bacilli.

Another modification in form which manifests itself upon the firmer serum is the appearance of round, deeply stained bodies, at or near the end of the usually slender bacilli, very rarely in the middle. These bodies, from 1.5 to 2 times the diameter of the rod, take a brilliant stain in carbol-fuchsin, contrasting with the more feebly stained, less compact rod in which they are imbedded. These bodies, which have a striking resemblance to spores, may be present in all the rods of any one preparation, or in a certain proportion only. They have been seen in Nasua, Sputum III, IV and VI. In the bovine cultures bodies of this nature are occasionally recognizable, but owing to the shortness of the bacilli they do not stand out distinctly. Of much more frequent occurrence in these cultures, often the only forms present, are short fusiform or spindle-shaped rods, the central swelling being also deeply stained, and perhaps corresponding to the terminal body of the human forms. These bodies have been seen by Ehrlich, Nocard and Roux, Metchnikoff, Cornil and Babes, Czaplewski and Straus. They have been variously interpreted as spores, and bacilli containing them have been successfully double-stained. Their significance remains unsolved. The most probable explanation is a degeneration or involution form. They may, however, represent some resting stage and correspond to the short, brilliantly stained bodies frequently seen in giant cells, and not recognizable as bacilli. In unstained films, mounted in water, they cannot be distinguished even with a very narrow pencil of light. An increased refrangibility cannot be made out.

Experiments to determine the length of time required at 60° C. to destroy both human and bovine forms have thus far not revealed any differences. The tests are reserved for a special publication.

A study of the relative resistance of the bacilli to drying has not yet been attempted.

Among the sputum cultures is one (No. 1) which grew so feebly on dog's serum that its cultivation was finally given up. The history of the case is briefly given in Table I. Since the comparative tests of the pathogenesis of this culture are very incomplete, I shall give briefly the

results of the inoculation of the sputum as evidence that the organism was in reality a tubercle bacillus.

June 27, 1896. Guinea-pig, weight 640 grammes, receives into abdomen 0.5 cc. of bouillon in which some sputum is suspended.

July 20. Weight 457 grammes. Animal rather dull, with coat slightly staring. Very little resistance to handling. Chloroformed.

A few subserous tubercles around point of injection. Omentum represented by a cylindrical mass of neoplastic tissue, about 1 to 2 cm. in diameter, firm, speckled with whitish dots indicative of necrosis. Tubercles on diaphragm and testicles. Liver spotted with small, irregular, not well-defined, yellowish necroses. Spleen slightly enlarged, with Malpighian bodies prominent. Bronchial and sternal glands enlarged, with central necrosis. Lungs free from visible tubercles.

The pathogenic power of this sputum seems to have been equal to that of most sputa examined. One guinea-pig of 672 grammes, inoculated precisely like the preceding, died 46 days after inoculation, with more pronounced lesions of the liver and spleen. A third guinea-pig of 370 grammes, which had received the same quantity subcutaneously, weighed a trifle more on the 49th day (390 grammes) than at the beginning. On this day it was chloroformed and examined. The typical ulcer with surrounding tubercles in the subcutis and a few tubercles in the omentum were seen. The liver was pale and fatty, the spleen enlarged to several times the normal size and uniformly permeated with grayish (one mm.) foci, but without necroses. The lungs were studded with small, partly necrotic tubercles. The bronchial and sternal glands were large, with necrotic centres.

The first culture showed somewhat slender but well-stained bacilli. In tubes subsequently inoculated, the bacilli multiplied almost exclusively in the condensation water, which became milky in appearance. In this the bacilli appeared in roundish clumps among cholesterin crystals. The bacilli were imbedded in a colorless material which formed the cohesive clump. The stained bacilli presented no unusual characters save in one culture (after about six months of total cultivation), in which fully one-half of all the quite slender bacilli appeared as series of transverse, apparently unconnected stained blocks, simulating streptococci. It is unfortunate that more animal tests were not made with this stock. As I was expecting a more vigorous culture from month to month, tests were postponed until conditions should approximate those of the other cultures. These were, however, not realized. The only test upon a rabbit is given in Table III., The cultivation was finally abandoned.

Of the other animal bacilli the cultures from swine were identical with the bovine cultures in morphological and other characters. Those of the horse and the cat were also either identical with the bovine bacilli or they very closely resembled them.

If we undertake to summarize the observations made with microscope and culture tube upon these bacilli, we somewhat hesitatingly formulate the following general statements:

1. Bovine and other animal bacilli (except *Nasua*, which is regarded as coming from man) grew less vigorously for a number of generations than the sputum bacilli. Sputum I is an exception and is probably an atypical form.

2. Bovine bacilli are much less influenced by certain modifications of the culture-medium.

3. Bovine bacilli tend to remain short; human bacilli are either more slender from the start or become so during cultivation.

PATHOGENESIS.

The most important means of demonstrating identity or divergence of characters of pathogenic bacteria is the test upon animals. This test is a purely physiological one, but in the application of experimental results from the domain of bacteriology to medicine, morphological characters must give way to physiological.

That there might be differences among tubercle bacilli from different species of mammals presented itself as a possibility to Koch and is referred to in his monograph. He, however, failed to recognize any differences worth mentioning. There are several reasons for this. In the first place, Koch was at that time endeavoring to prove to the world that the tubercle bacillus is the cause of tuberculosis. Minor details received no attention because of the great and difficult task immediately before him. In the second place, the course of tuberculosis is essentially a function of the number of bacilli introduced. Variations in the dose result in corresponding variations in the length of the disease, in its final termination, and in the extent and distribution of the lesions. This fact Koch himself recognized in discussing the results following the intra-ocular inoculation of varying quantities of culture material in rabbits. He did not, however, attempt to adjust

carefully the quantity to be injected or to determine the effect of varying quantities of bacilli upon any of the species used by him. Curiously enough, no one following him seems to have considered it worth while to extend his work by more accurate comparative tests. That such work is likely to be fruitful the following experiments amply demonstrate.

Guinea-pigs. The inoculation of this species with tubercle bacilli from the various cultures brought out few well-defined differences. The great susceptibility of guinea-pigs to tubercle bacilli is responsible for the general acceptance of a common level of pathogenic power for all mammalian tubercle bacilli. This tendency to unify physiological characters because of a more or less uniform action upon very susceptible species has led to frequent failure on the part of experimenters to recognize important variations among species of bacteria, variations probably having much influence upon the course of the disease contracted in the natural way. There are, however, certain differences in the action of bovine and sputum bacilli upon guinea-pigs which enable us to recognize even in these very susceptible animals the much greater virulence of bovine bacilli. It is probable that by a graded dosage of tubercle bacilli more marked differences might be brought out than I am in position to present.

It has already been stated that all the cultures in hand had been obtained by inoculating the tuberculous tissue or the sputum into guinea-pigs and isolating the bacilli from them. To obtain the bovine cultures, a small bit of tissue was placed in a subcutaneous pocket in the flank. For sputum bacilli the sputum was shaken up in sterile bouillon and the dilution injected into the abdominal cavity. In these preliminary experiments differences presented themselves between the two kinds of material. Although the sputum, as a rule, contained very many tubercle bacilli and the bovine tissue very few, yet the subcutaneous injection of sputum led to such a chronic disease, with comparatively slight lesions and a tendency to reparative changes in the liver, that the animals were very unpromising subjects for culture when chloroformed.* Hence the intra-abdominal inoculation in most cases. On the other hand, the disease due to bovine tissue placed in the subcutis never failed

* These statements apply only to guinea-pigs weighing 400 grammes and above.

to produce extensive lesions, with indications of certain death in 4 to 6 weeks, which was anticipated by chloroform when cultures were to be made. Leaving this preliminary series of inoculations, we will pass to those made with definite doses of tubercle bacilli in suspension.

The suspension was made by thoroughly rubbing the growth from blood serum upon the inside of dry sterile test tubes with a heavy spatula-like platinum wire. This procedure results in the breaking up of clumps and the coating of the tube with masses of bacilli. Bouillon was then poured in and the thoroughly stirred suspension allowed to stand for several hours until all coarser particles had subsided. The resulting suspension was diluted, if necessary, until its density was nearly equivalent to that of a bouillon culture of typhoid bacilli 20 to 24 hours old, and nearly as homogeneous. More accurate dosage might be obtainable by weighing the moist growth before suspending it in bouillon; but inasmuch as the weighed quantity may not all be brought into a finely divided state—a matter of importance in the intravenous injection of rabbits—the method adopted is probably less troublesome and equally accurate.

Of this suspension the usual dose, both for subcutaneous and intra-abdominal inoculation, was 0.5 cc. An examination of the dates in the various tables will show that many of the inoculations upon rabbits, guinea-pigs and cattle were made with the same suspension on the same day.

The guinea-pigs employed were all rather large, weighing, with few exceptions, from 400 to 700 grms. They had all been used in diphtheria antitoxin tests one to three months before. The rapid increase in weight was evidence of no disturbance of health at the time of inoculation. It is of importance to bear in mind that the animals were large, because the progress of tuberculosis differs essentially in growing and adult guinea-pigs, the latter manifesting a far greater resistance than the former, a resistance expressed by well-marked reparative or cirrhotic changes in the liver. The differences to be pointed out in the bovine and sputum series may possibly be nearly wiped out by the use of guinea-pigs weighing only 250 to 300 grms.

The disease induced by the intra-abdominal injection was so rapid in its progress for both bovine and sputum cultures that the virulence of both races appears nearly the same.

The shortest period between inoculation and death, when 0.5 cc. of the suspension was injected, is 7 days, the longest 21 days, with one

exception, that of Sputum VI, which was 38 days. In other cases the period was prolonged because of smaller doses. For Bovine I (0.2 cc.) it was 33 days; for Nasua (0.3 cc.) 41 days. If we take the largest series in which the injections were made at the same time and in which the conditions were identical—the series comprising cultures of Sput. IV, V, VI and of Bovine III, IV, V—we find the periods elapsing between inoculation and death to be respectively 21, 11, 38 for the sputum and 7, 9 and 10 days for the bovine cultures.

The disease due to the intra-abdominal injection of tubercle bacilli in the quantity used is peculiar in this, that there is always found after death a large quantity of an opalescent fluid in both pleural sacs. This fluid greatly compresses the lungs and is probably the immediate cause of death. When the disease is prolonged beyond 3 weeks, the pleural effusion is absent. A careful histological study of the various organs in this most acute form of the inoculation disease, which will probably furnish the explanation of the effusion, is still to be made. In the abdominal cavity there is, as might be expected, a general inflammation of the peritoneum, associated with some fibrinous exudation and usually a little viscid fluid, when the disease does not last quite ten days. Any prolongation beyond this brings a dense eruption of tubercles into view. The omentum is in all cases represented by a cylindrical mass of fused tubercles.

If we turn to the subcutaneous inoculations, the difference in pathogenic power is more clearly brought out.

In the same series just quoted, of the three sputum guinea-pigs, one died 44 days after inoculation, the others were chloroformed respectively 77 and 100 days after inoculation; the first having lost but little in weight, the second being a trifle heavier, but having extensive pulmonary disease. The bovine guinea-pigs died in 51, 41 and 40 days respectively. The sputum guinea-pig, which succumbed in 44 days, weighed at the start 130 grms. less than the lightest bovine guinea-pig. The lesions were slight, the lymph glands only moderately enlarged, the foci in the lungs not visible to the naked eye. The spleen was not enlarged. The liver was mottled with very many grayish specks. The microscopic examination showed lesions but slightly advanced and reparative changes with proliferation of bile-ducts in progress. In general, the tuberculous lesions in this animal did not warrant so early a death.

The histological examination of the tissues of the infected guinea-pigs was restricted to the lungs and the liver of those inoculated simultane-

ously under the skin with cultures of Sputum IV, V and VI,* and of Bovine III and V. These organs were chosen because of their peculiar behavior towards the infection. The liver is invaded first, and under favorable conditions cirrhotic changes may begin which in some cases apparently lead to an elimination of the disease, with considerable shrinking of the organ and furrowing of the surface. In the lungs the disease begins later, and if the animal survives the first 6 weeks, may grow steadily until most of the organ is involved.

Pieces of tissue hardened in Zenker's fluid or alcohol were cut embedded in paraffine. The sections, fastened to the slide with Mayer's albumen and water combined, were stained with hæmalum and eosin or picro-acid-fuchsin, or with eosin and methylene-blue. In some cases Weigert's fibrin stain was resorted to. All were stained for tubercle bacilli in carbol-fuchsin, differentiated in 1 per cent acetic or decolorized in 4 per cent sulphuric acid, and stained subsequently in Löffler's methylene-blue. The stain for tubercle bacilli was successful in the tissues hardened in Zenker's fluid. Even in the sections stained according to Gram-Weigert, the bacilli appeared distinctly, but in a peculiar form, often simulating short streptococci with the individual cells rather well separated, or series of short blocks with spaces between them. Here the stain fastens itself only upon certain segments of the bacillus, leaving the connecting elements entirely unstained. The part selected evidently stands in no relation to the bulging, deeply stained, spore-like bodies referred to above as occurring in cultures.

In discussing the histological changes induced by tubercle bacilli in the various animals inoculated, it has been my determination to steer clear of all controversial questions relating to the histogenesis of the tubercle or of the elements composing it, as being foreign to the scope of this etiological study. Only those features will be very briefly referred to which show pronounced variations and which serve to point out differences in the action of the bacilli.

In the liver of the inoculated guinea-pig, the proliferative lesions present in all cases examined consist of aggregations of the characteristic epithelioid cells, among which, here and there, scattering nuclei appear richer in chromatin, and which for convenience are designated lymphoid elements. The lesions, situated chiefly in the interlobular tissue, are surrounded with a wide or narrow zone of proliferating bile-ducts, which appear as single or forked lines or in the form of meshes. There is noth-

* Spleen in place of liver in this case, the latter having been rejected by mistake.

ing in the microscopic appearance of the liver tubercles to serve as distinguishing marks, excepting the much more extensive involvement of the liver tissue and the much greater abundance of tubercle bacilli in the bovine cases. In the latter there are added to these lesions those of another character, the simple necrosis of cell territories ushered in by a fading out of the nuclei and a deep stain with eosin. These areas probably represent the yellow cheesy areas so common, and often very large, in young tuberculous guinea-pigs. They are readily distinguishable with the naked eye from the grayish proliferative changes going on in the interlobular tissue. In the sputum cases the very rare areas of this character seen were very small.

In the lungs, the lesions are as a rule perivascular and peribronchial. There are no essentially distinctive features here, excepting the very great abundance of tubercle bacilli in the bovine cases. In Bov. III the bacilli are also found in large numbers in cell detritus occupying alveoli and small bronchi. In Sputum VI many alveoli in the periphery of the foci are nearly filled out with multinucleated cells resembling giant cells closely, but their contents are only pigment particles. In Bovine V similar cells are present, though in small numbers. These, as well as other cells in the alveoli, each contain several bacilli.

The action of tubercle bacilli from the pig, the horse and the cat upon guinea-pigs was nearly the same as that of the bovine cultures, and needs no special discussion here.

With this hasty, imperfect sketch we may conclude the notes on the tuberculosis of guinea-pigs induced with cultures, by simply adding that the bovine bacilli studied are more virulent than human bacilli, although, owing to the great susceptibility of this species, the differences are not likely to impress us unless we give much attention to experimental details. These differences may be summed up as consisting in the more rapid death of all guinea-pigs inoculated with bovine bacilli, the greater extent of the lesions and the far greater abundance of tubercle bacilli in them.

In Table II will be found, in part, the basis for these conclusions.

TABLE II.—GUINEA-PIGS.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days.	Dose in cc.	Mode of inoculation.	Initial weight in grammes.	Final weight.	Result.
Sputum I	No inoculation of cultures.	———	(Of sputum suspension) June 27, 96.		0.5	intra-abd.	672	430	Dies Aug. 11–12.
					0.5	sub-cut.	370	390	Chloroformed August 15 (active).
					0.5	intra-abd.	640	457	" July 20 (sick).
" II	3 mos. 3 days.	5th	Feb. 24, 97.	24	0.5	sub-cut.	570	520	Chloroformed April 19.
					0.5	intra-abd.	600		Dies March 6–7 (10–11 days).
	5 " 10 "	8th	May 1, 97.	9	0.5	" "	440	312	" May 13 (13 days).
" III	1 " 19 "	2d	April 3, 97.	18	0.5	" "	525		" April 12–13 (9 days).
	2 " 17 "	4th	May 1, 97.	9	0.5	" "	435	245	" May 17 (17 days).
" IV	2 " 16 "	2d	May 1, 97.	18	0.5	" "	705		" May 15–16 (15 days).
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	sub-cut.	372	275	" Oct. 9.
	6 " 10 "	7th	Aug. 26, 97.	9	0.5	intra-abd.	507		" Sept. 16–17 (21 days).
" V	1 " 19 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	458	402	Chloroformed Dec. 3.
					0.5	intra-abd.	517		Dies Sept. 5–6 (11 days).
" VI	1 " 12 "	3d	Aug. 26, 97.	9	0.5	sub-cut.	420	431	Chloroformed Nov. 11, extensive lung disease.
					0.5	intra-abd.	478	338	Dies Oct. 3 (38 days).
" VII	1 " 10 "	2d	March 1, 98.	16	0.5 *	sub-cut.	323	360	Dies May 14–15.
					0.5 *	intra-abd.	332	222	Dies March 18–19 (18 days).
Bovine I	15 " 19 "	14th	Mar. 20, 96.	16	0.5	sub-cut.	405	?	Chloroformed May 14 (failing).
					0.2	intra-abd.	478		Dies April 22 (33 days.)
" II	2 " 27 "	3d	Feb. 24, 97.	24	0.5	sub-cut.	580	?	" Mar. 24.
					0.5	intra-abd.	645	?	" Mar. 3–4 (7 days).
	5 " 3 "	5th	May 1, 97.	9	0.5	" "	505	?	" May 13 (13 days).
" III	4 " 7 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	533	400	" Oct. 17–18.
					0.5	intra-abd.	624	?	" Sept. 2–3 (7 days).
	9 " 27 "	8th	Feb. 15, 98.	17	0.5	" "	581	468	" Mar. 2 (15 days).
" IV	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	635	400	" Oct. 6–7.
					0.5	intra-abd.	533	?	" Sept. 4–5 (9 days).
" V	4 " 3 "	5th	Aug. 26, 97.	9	0.5	sub-cut.	504	336	" Oct. 5.
					0.5	intra-abd.	490		" Sept. 5–6 (10 days).
" VI	2 " 18 "	3d	Mar. 15, 98.	13	0.5 *	sub-cut.	406		Dies May 2–3.
					0.5 *	intra-abd.	423		Dies April 7 (23 days).
Nasua	19 " 22 "	18th	Mar. 20, 96.	16	0.75	sub-cut.	455	?	" April 26, probably chronic kidney disease.
					0.3	intra-abd.	505	?	" May 1 (41 days).
Swine I	2 " 6 "	14th	July 14, 96.	12	0.5	sub-cut.	525	?	" Aug. 7.
					1.0	intra-abd.	525		" July 25–26.
	11 " 23 "	16th	May 1, 97.	13	0.5	" "	450	300	" May 15 (14 days).
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	sub-cut.	395		" Aug. 19–20.
					0.75	intra-abd.	415	290	" Aug. 7 (14 days).
Cat I	4 " 15 "	5th	July 24, 97.	18	0.75	sub-cut.	424	290	" Aug. 28–29.
					0.5	intra-abd.	427	?	" Aug. 8 (15 days).

* Dilution one-third to one-fourth of that hitherto used.

Rabbits. The inoculation into the anterior chamber of the eye has been the most widely practised method. This seems to have been followed by variable results, whether due to the number of bacilli injected, the source of the bacilli or the amount of injury inflicted on the eye.

Koch injected tubercle bacilli from various sources into an ear-vein. The bacilli were suspended in blood-serum and the suspension passed through fine gauze to eliminate coarser particles. 4 rabbits were inoculated in this way with bacilli from a monkey, 3 with bacilli from a case of phthisis and 3 with bacilli from cattle. The rabbits all succumbed in from 18 to 31 days, excepting one, which was killed on the 38th day. In another experiment a culture from a case of lupus and from a monkey were tested on rabbits. Death ensued in 2 to 3 weeks. The weight of the rabbits and the density of the suspensions used are not given, so that comparisons are impossible. Moreover, the medium used—liquid serum—may not be entirely indifferent. Straus* mentions the slight effect produced upon rabbits by cultures of human tubercle bacilli.

In the present work, the injection of tubercle bacilli into an ear-vein was used at first tentatively, then, when sharp distinctions between bovine and human cultures were made manifest by this procedure, it was continued.

Rabbits weighing from 1300 to 2200 grms. were employed. The suspension was prepared as already described, and for most of the primary tests 0.5 cc. was injected. By referring to Table III it will be seen that for the primary test of the last pair of cultures, Bovine VI and Sputum VII, the suspensions were $\frac{1}{3}$ to $\frac{1}{4}$ as dense and the dose was doubled. The other cultures of which larger doses were injected are Sputum I, Nasua and Cat I (0.75 cc.), and Swine I (1 cc.). More recent tests indicate, however, that any variation in the dose as slight as these has but little influence in modifying the duration of the disease.

As far as practicable, the tests were made as soon as the purity of the cultures was beyond doubt, in order to reduce to a minimum the factor of artificial attenuation. This seems to have been on the whole incon-

* La tuberculose et son bacille, 377.

siderable.* An effort was also made to test the cultures in groups as far as possible, in order to furnish identical conditions. These groups are Bovine I and Nasua, Sputum II and Bovine II, Sputum III and IV, Sputum I and IV, Horse I and Cat I, Sputum V, VI and Bovine III, IV and V. Sputum VII and Bovine VI may be considered a group, though the inoculations were not made simultaneously, owing to an accident. The ages of the different groups of cultures varied from a minimum of 9 to a maximum of 24 days. The total age of the cultures likewise varied at the date of the first test from 1 month and 10 days to 9 months and 25 days. These differences, which were largely counterbalanced by the group inoculations, cannot be dwelt upon here, as the table contains all the information needed for the reader to form his own opinion. It should be stated, however, that the heaviest rabbits of any group were chosen for the bovine cultures. After the inoculation the rabbits were handled very little, in order that the conditions might remain as uniform as possible. For this reason I have no temperature records, but hope to obtain them from a special series of experiments.

The results may be classed as gross or those manifest on ordinary observation and those deducible from a microscopic examination of the diseased tissues and organs. The gross results show a sharp line of demarcation between the bovine and the human cultures. While all the rabbits inoculated with the former succumbed in from 17 to 21 days, of the rabbits inoculated with the latter only one succumbed in 35 days. The others were chloroformed after periods ranging from 1 month and 24 days to 3 months and 18 days. At the time they were killed all but one had increased in weight, some but little, others much.

The rabbits inoculated with bovine bacilli manifested in most cases more or less dyspnœa at the end of 8 or 10 days. The lesions were practically the same in all, with slight minor modifications. The lungs, as the first and chief recipients of the injected bacilli, were in a state of expansion, which in some cases was extreme. All lobes were densely sprinkled over with small grayish tubercles, varying from mere specks to those 1 mm. in diameter. The liver was usually mottled, indicating fatty changes, and in about half the cases it was permeated with barely visible grayish points. The spleen was enlarged to 2 or 3 times its normal size

* See Table III, Bovine culture I.

and as a rule permeated with minute tubercles. In the kidneys the visible tubercles were scarce. In 3 cases they were seen in the heart muscle, in several in the follicles composing Peyer's patch at the ileocæcal valve.

The sputum rabbits, with one exception, behaved quite differently from the bovine cases. A certain number showed signs of illness after 7 or more days. They were quiet, somewhat drowsy and ate little, but manifested no dyspnœa. This condition lasted one or two weeks, after which recovery took place. A certain number did not show this transitory depression. It is probable that with all, during the first month, there was loss of weight, for subsequent weighings showed no gain upon the initial weight. At the time they were chloroformed, they had all more than recovered in weight. The lesions in these rabbits were in many respects quite different from those of the bovine rabbits. The rabbit which received the culture marked Sputum I did not show any recognizable lesions. The difficulty experienced in the attempts to cultivate this bacillus leaves the interpretation of this case somewhat doubtful, although the microscopic preparation from the feeble culture used showed well-stained bacilli. The lesions of the other cases were quite uniform in character.

In all, the lungs contained tuberculous foci sprinkled over the various surfaces from 1 mm. to 1 cm. apart. They did not seem to interfere functionally, for the lungs collapsed normally when the thorax was opened. The tubercles frequently projected somewhat above the surface in the collapsed state of the organ. In the fresh condition the foci were observed as minute dots, barely visible owing to their translucency, as larger foci, 2 mm., in rare cases 3 to 4 mm. in diameter, also translucent excepting the centre, which was occupied by a minute opaque point, and as more diffuse infiltrations of a translucent appearance mainly at the sharp lateral margin of the lobes. These different forms of lesions were usually found associated in the same organ, the largest in the animals kept alive longest. Necrosis was an inconspicuous feature, whereas the translucency was often so perfect as to conceal the small lesions from the eye. When placed in alcohol, these became opaque and visible.

The liver usually contained minute tubercles scattered sparsely over the organ. The spleen, only very slightly larger than normal, was affected to nearly the same degree. The kidneys came next to the lungs in the extent of the lesions. Sprinkled sparingly over the cortex were opaque whitish foci from one to two mm. in diameter. When the organ was incised, these round foci, in many cases, corresponded to radial whitish lines and bands, extending from the periphery towards the pelvis and usually stopping within the medullary portion near its base. More

rarely small whitish foci were observed in the walls of the stomach or the large intestine. The lymph nodes were free excepting in one case, in which the node associated with a tuberculous kidney was involved.

In all cases inspection of the affected organs revealed no degenerative changes. They were evidently not disturbed by the focal lesions, an observation strengthened by the increased weight of the animals when sacrificed. The single rabbit which died 35 days after inoculation had received Sputum culture IV. It is worth while to note as a highly probable explanation of this one exception that the rabbit, after inoculation, escaped from the basket in which it was being carried and had to be chased for a long time in the animal room before it could be penned. I at once anticipated some modification of the disease, owing to the different distribution the bacilli would undergo under the influence of muscular exertion. This anticipation was realized. The rabbit after a week became quiet and remained dull and drowsy, without any change in respiration. On the day before death it sat in a crouching position, the hind feet drawn forward under the body, the eyes partly closed. After death its weight was 922 grammes, the initial weight having been 1620 grammes. During the latter weeks a purulent ophthalmia developed in the left eye, and shortly before death there was a slight discharge and beginning opacity of the cornea in the right eye.

In the liver, lungs and kidneys there were minute, translucent tubercles, scarce in the lungs and kidneys, more abundant in the liver. The lesions, found in sections of hardened tissue to be relatively very immature, could not explain the rapid emaciation and death, as they were less extensive than those of the surviving rabbits, unless we assume that the different, more general distribution caused a more rapid breaking up of the bacilli and diffusion of the toxines. Another hypothesis would be the presence of foci in the central nervous system, which was not examined. A second trial upon a rabbit 2 months later, with conditions as nearly the same as possible, proved no exception to the general rule. The rabbit showed but little disturbance, and was chloroformed after 2 months and 9 days. The lesions found were like those induced by the other sputum bacilli.

In order to probe still farther this difference between bovine and human bacilli, the lungs of all but a few rabbits were subjected to a microscopic study. Here the foci may all be considered primary and produced under like conditions. The lesions as defined in fixed and hardened tissue by the microscope in the bovine cases were characterized by rapid necrosis of the tubercle and the presence of very large numbers of tubercle bacilli. This necrosis was evident in the fresh organs from

the marked opacity of the comparatively young tubercles. The tubercle itself at the stage in which it came under observation varied slightly from case to case. It consisted of a necrotic centre, absent only in the smallest foci, outside of which a zone of epithelioid cells was distinguishable. This zone was in several cases bounded by a peripheral one of cells having deeply stained nuclei and probably lymphoid. These latter cells were also intermingled with the epithelioid ones. The central necrosis appeared in two forms; it either was characterized by the presence of a very dense, deeply stained mass of nuclear fragments, or else these were present only to a slight degree and the centre had a more rarefied aspect. In the latter case it was evident that the necrotic mass consisted of the bodies of epithelioid cells and the few nuclei present. In the former the source of the great mass of nuclear detritus remains in doubt. These dense nuclear masses were present in abundance in Bov. I and III. In Bov. II and IV they were not quite so conspicuous; in Bov. V least so. It is worthy of note here that Bov. I and III are the two cultures which proved rapidly fatal to cattle inoculated with them, as will be shown farther on.

In the series of rabbits inoculated with human bacilli, the lungs present quite different conditions. The lesions evidently develop very slowly, for in the one rabbit which succumbed after 35 days, the tubercles were very small, translucent, the bulk microscopic in size. With the continued life of the rabbit they grow larger. The microscopic picture differs from that of the bovine series in the rarity of necrosis and in the comparative scarcity of tubercle bacilli. The tubercles consist of aggregations of large epithelioid elements which only in the centre of the larger foci show necrosis. These epithelioid foci may be rarely enclosed by a zone of round cells. Giant cells are present, but as a rule in very small numbers. In the Sputum III rabbit they were exceptionally numerous and very large. The contiguous alveoli may be occluded with patches of epithelioid cells not to be confounded with desquamated epithelium.* Tubercle bacilli are found only in the necrotic centres, either in very small numbers or more abundantly. In some cases they stain feebly. In all cases they are somewhat longer than the bovine bacilli in the same situation.

In addition to the foregoing inoculation tests, made as far as practicable with equal doses, a few others were tried under slightly modified

* The lesions in rabbits due to sputum bacilli resemble in many respects those produced by Prudden with boiled and washed tubercle bacilli in the same species, if we exclude the slight progressive tendency of the disease due to the living forms. See *N. Y. Med. Journal*, 1891, i, 637.

conditions. A large rabbit (No. 20), weighing 2048 grms., received into the abdominal cavity the usual dose, 0.5 cc. of the standard suspension. Death ensued 26 days later. The walls of the abdomen and of the large intestine were densely studded with minute tubercles, the liver permeated with them. The lungs also were densely beset and penetrated with minute translucent tubercles. They did not differ appreciably from the lungs after intravenous inoculation. This mode of inoculation thus proved almost as promptly fatal as the intravenous injection. The same suspension of tubercle bacilli used upon the preceding rabbit was diluted with 9 volumes of bouillon, and 0.5 cc. injected into an ear-vein of a rabbit (No. 27) weighing 1932 grms. The rabbit thus received one-tenth the usual dose. In this animal the symptoms and duration of the disease and the lesions were nearly the same as those of the rabbits inoculated with the horse culture to be described later. The rabbit showed evidences of disease after two weeks, with slowly increasing dyspnœa. On the 34th day it was chloroformed, as it would probably have lived but one or two days longer. The extreme relative virulence of the bovine bacilli is thus put into a clear light.

A similar experiment was made with Sputum culture III. One rabbit received an equivalent of the usual dose, another one-tenth of this on March 1st. Both are still alive (July 20), and the initial weights of 1707 and 1672 grms. are now 1558 and 1986 grms.

It has already been stated that the Nasua culture, presumably derived from sputum, produced neither symptoms nor visible lesions in a rabbit. The swine and the cat culture, however, produced lesions practically identical with those due to the bovine cultures.

The horse culture takes a position midway between bovine and human cultures, so far as the rabbit inoculations show. These, therefore, deserve a more detailed description. The first rabbit, treated like all the rest, remained apparently unaffected for a month, although the weight had fallen slightly from 1505 to 1477 grms. In the second month respiration became somewhat slower and grew gradually more and more labored, so that the animal had to be chloroformed just two months after inoculation. The weight had fallen to 1232 grms. The lesions were macroscopically unlike those in any other rabbit of the series. In the first place, the lungs failed to collapse even slightly. They are densely beset and permeated with tubercles of a grayish, feebly translucent appearance, the majority about 1.5 to 2 mm. in diameter, coalescing into patches 4 and more mm. in diameter. Minute tubercles are also visible among the larger ones. The latter may have a minute, opaque, necrotic centre. The liver is apparently free from tubercles, but the slightly

enlarged spleen is beset with sharply outlined, slightly projecting grayish tubercles 1 mm. in diameter. The kidneys are affected in a manner similar to those of the sputum rabbits, but the foci are larger. There are scattering tubercles in the walls of the small and large intestines, but none in the walls of stomach. A second rabbit, inoculated 6 months later, was chloroformed after 40 days on account of severe dyspnœa. The course of the disease and the lesions were like those of the first rabbit. A noteworthy peculiarity of these cases is the presence of tuberculous foci 2 to 4 mm. in diameter in the superficial axillary and popliteal lymph nodes. These were not seen in any of the sputum rabbits, but were produced by retarding the bovine disease in a rabbit. The intermediate position of this horse culture is well illustrated by this experiment referred to above, in which a dilution of bovine tubercle bacilli, equivalent to one-tenth of the usual density employed, produced manifestly the same effect upon rabbits as the horse culture. In other words, the pathogenic power of the bovine culture as measured upon rabbits was 10 times that of the horse bacilli. Further trials will, perhaps, enable us to give a rough arithmetical ratio between the pathogenic power of the bovine and the sputum cultures as affecting adult rabbits. I do not, however, consider the difference between these races to be solely quantitative.

A comparison of the lesions produced by these three races was attempted by a histological examination of the lungs, spleen, liver and kidneys of three rabbits:

No. 16. Wt. 1527–1617: Sput. V: Chloroformed 3 months 7 days after inoculation.
No. 14. Wt. 1540–1232: Horse I: " 2 " 14 " " "
No. 19. Wt. 1443– Bov. V: Died 20 days after inoculation.

I refrain from giving the detailed account of the lesions in each organ and limit myself to the following summary:

Sputum V. Homogeneous epithelioid cell foci of small size, with very little central necrosis and very few tubercle bacilli. In the lungs and kidneys these foci have lymphoid cell mantles, in the liver well-defined connective tissue capsules.

Horse I. Large foci in lungs, where they are so numerous as to coalesce; some large foci in kidneys; smaller ones in the spleen. In the liver the foci are represented simply by one or several large giant cells, situated within the lobules. Foci large in subcutaneous lymph nodes and Peyer's patch. Necrosis of the foci quite extensive in all the organs excepting the lungs, where it is slight. Tubercle bacilli fairly abundant in all foci.

Bovine V. Large numbers of epithelioid cell foci in lungs, liver and spleen. In the latter epithelioid cells are also abundant throughout the pulp. Foci small in kidneys. Necrosis under way in lung foci. Tubercle bacilli very abundant in all foci.

Taking into consideration the time during which these lesions developed, we may epitomize the observed differences as follows:

In the first rabbit, very gradual development of the tubercles, with suppression of the bacilli and reparative changes leading to a stationary condition of the disease. In the second rabbit, continuous but not very rapid development of tubercles in the various organs. In the kidney and spleen evidences of limitation of the foci. No evidence that bacilli are repressed. In the third rabbit very rapid appearance of the foci, with unchecked multiplication of the bacilli and rapid onset of necrotic changes.

As a matter of some interest there may be briefly mentioned certain lesions of the eye in three of the sputum rabbits. In rabbit No. 7, inoculated with Sputum culture IV, which animal was the only one of all the sputum rabbits to succumb (in 35 days), a purulent ophthalmia appeared in the left eye some time before death. Soon after a slight cloudiness of the cornea of the other eye was noticed. A discharge had started in this shortly before death. I did not associate these lesions directly with tuberculosis, although later cases make it probable that the purulent discharge was secondary to tuberculosis of other parts of the eye. At the autopsy I examined the discharge for tubercle bacilli, but found no acid-resisting bacilli.

The second rabbit (No. 12) inoculated with a culture from the same source first called my attention to this localization of the tubercle bacilli. In this animal there was noticed some weeks after the inoculation, a slight nodular thickening of both lids of the right eye, which slowly grew more conspicuous. Vision became impaired by the enlargement and gumming together of the lids, so that the rabbit used the other eye only. When this animal was chloroformed, 3 flattish, disk-like, firm excrescences were found on the sclera at the margin of the cornea, about 90° apart. Of these, as well as of the nodulated lower eyelid, sections were made, and both lesions proved to be tuberculous. In both the tubercle bacilli were sparingly present. The neoplasm in the eye-ball had involved nearly the whole thickness of the sclera. In the eyelid the multiple foci were external to the lobules of the Meibomian glands. In still another animal eye lesions were found. About 2½ months after rabbit No. 16 had been inoculated with Sputum culture V, the lids of the left eye were observed to be gummed together. A pasty, yellowish

exudate was found under the lids and the cornea was slightly opaque. About 3 weeks later the animal was chloroformed and faint whitish spots were seen in both eyes, presumably on the iris. Sections of the eye hardened unopened in Zenker's fluid confirmed this supposition. Small epithelioid tubercles were found in the vascular stroma of the iris as well as in one ciliary body. Linear infiltration of the cornea was observed, starting from the most affected side and extending to the middle of the cornea. In its course several sections of vessels were found. A similar infiltration had begun from the opposite side. In the foci tubercle bacilli were very scarce. The infection probably came through the blood, as infection from the point of injection on the ear is hardly supposable, since the lesions here are of the slightest character. The animals had been kept separated, and could not have acquired the infection from the exterior. The eye of the rabbit thus seems to be a very vulnerable organ, and earlier experimenters have, in a way, chosen by accident that organ in this species most likely to give positive results.*

The divergences noticed when rabbits weighing between 1300 and 2200 grammes are inoculated into an ear-vein with 0.5 cc. of a well-clouded suspension of tubercle bacilli in bouillon are as follows:

1. Death of the bovine cases in 17 to 21 days.
2. Rapid evolution and necrosis of the pulmonary tubercles, with very great increase of the tubercle bacilli in them.
3. Death of the sputum rabbits (one explainable exception) did not ensue, but after $1\frac{1}{2}$ to $3\frac{1}{2}$ months the original weight had been more or less exceeded.
4. The pulmonary tubercles in the sputum rabbit developed very slowly, with very little tendency towards necrosis. The bacilli were present in very small numbers only.
5. The swine and cat cultures are to be classed with the bovine, the horse culture stands intermediate.

* The eyes of Sputum rabbit VII were affected in the same manner.

TABLE III.—RABBITS.

Culture.	Total age of artificial culture.	Number of transfer.	Date of inoculation.	Age of culture used, in days.	Dose in cc.	Mode of inoculation.	Designation of rabbit.	Initial weight in grammes.	Final weight.	Chloroformed after	Died in
Sputum I	10 mos. 27 days.	2nd	June 16, 97.	61	0.75	ear-vein.	17	1470	1681	2 mos. 3 days.	
" II	3 " 3 "	5th	Feb. 24, 97.	24	0.5	" "	6	1380	1645	1 " 26 "	
	1 " 19 "	2nd	April 3, 97.	18	0.5	" "	8	1790	1830	2 " 8 "	
" III	13 " 17 "		Mch. 1, 98.	16	1.0‡	" "	28	1707		Alive July 20; weighs 1558.	
	13 " 17 "		" " "	16	1.0*	" "	26	1672		Alive July 20; weighs 1986.	
" IV	1 " 18 "	2nd	April 3, 97.	18	0.5	" "	7	1620	922		1 mo. 5 days.
	4 " ——	5th	June 16, 97.	12	0.5	" "	12	1620	1722	2 mos. 9 days.	
" V	1 " 19 "	3rd	Aug. 26, 97.	10	0.5	" "	16	1517	1617	3 " 7 "	
" VI	1 " 12 "	3rd	Aug. 26, 97.	10	0.5	" "	18	1305	2061	3 " 18 "	
" VII	1 " 10 "	2nd	Mch. 1, 98.	16	1.0†	" "	29	1882	1788	3 " 8 "	
Bovine I	15 " 19 "	14th	Mch. 20, 96.	16	0.5	" "	3	2279	——		17 days.
	37 " 24 "	38th	Jan. 25, 98.	12	0.5	" "	24	1795	——		22 "
" II	2 " 27 "	3rd	Feb. 24, 97.	24	0.5	" "	5	1360	——		17 "
" III	4 " 7 "	5th	Aug. 26, 97.	10	0.5	" "	15	1775	——		21 "
" IV	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	17	1607	——		15 "
	9 " 23 "	9th	Feb. 15, 97.	17	0.5	abdomen.	20	2048	——		26 "
	9 " 23 "	"	" " "	17	0.5§	ear-vein.	27	1932	1416		Killed on 34th day, very severe dyspnœa, probably within 24 hours of time of death.
" V	4 " 3 "	5th	Aug. 26, 97.	10	0.5	" "	19	1443	——		20 days.
" VI	2 " 18 "	3rd	Mch. 15, 98.	13	1.0†	" "	21	2353	1877		Chloroformed on 22d day; dying.
Nasua	19 " 25 "	18th	Mch. 20, 96.	16	0.75	" "	2	1800	fat condition wt. ?	1 mos. 24 days.	
Swine I	2 " 6 "	4th	July 14, 96.	12	1.	" "	1	2000 (?)	——		17 days.
Horse I	5 " 4 "	6th	July 24, 97.	18	0.5	" "	14	1540	1232	2 " 4 "	
	11 " 5 "	11th	Jan. 25, 98.	12	0.5	" "	25	1653	——	1 " 9 "	
Cat I	10 " 17 "	5th	Jan. 25, 98.	18	0.75	" "	13	1320	——		20 "

* Dilution about one-twentieth of the standard. † Dilution about one-fourth of the standard. ‡ Dilution about one-half of the standard. § Dilution about one-tenth of the standard.

Gray Mice. White mice have been pronounced quite insusceptible by Koch. Of 5 inoculated subcutaneously with a culture from the monkey, 12 with a culture from miliary tuberculosis, only one of the first set showed a few grayish nodules in the lungs. On the other hand, field-mice were found quite susceptible.

Two parallel tests were made upon gray house-mice with cultures of Bovine V and Sputum VI. At the time control inoculations of the suspensions used were not made upon guinea-pigs, and subsequent developments made it doubtful whether Bovine V was alive. This part of the experiment is therefore omitted from the table given below and a later experiment with Bovine VI substituted. The suspension used in this latter test was employed simultaneously upon 2 guinea-pigs and a rabbit with the usual success.

In the first bovine test the 4 mice inoculated, 2 subcutaneously and 2 into the abdomen, all died, the first on the 8th and the last on the 37th day, without any lesions recognizable with the naked eye. In the cases of the 4 mice inoculated with the sputum culture, death ensued between the 8th and the 40th day. In one of these the lungs contained a number of whitish, rather firm, nodules, 1 to 2 mm. in diameter. In sections these were found to consist of completely necrotic foci, enveloped by a dense, broad zone of pneumonic infiltration, within which all alveoli were filled with nuclear debris. A striking feature were the immense numbers of rather slender, mainly beaded tubercle bacilli, which occupied not only the necrotic centre, but in equal abundance the pneumonic periphery. This case proved the vitality of the sputum culture, and the test is therefore inserted in the table below.

TABLE IV.—Gray Mice.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Dose and mode of injection.	Results.
Sputum VI	5 mos. 27 days.	6th	24 days	Mouse 1, 0.5 cc. subcut...	Dies in 34 days, no tuberculosis.
				" 2, 0.5 cc. " ...	Dies in 40 days, no tuberculosis.
				" 3, 0.25 cc. abdomen.	Dies in 8 days, no tuberculosis.
				" 4, 0.25 cc. "	Dies in 34 days, tubercles in lungs.
Bovine VI	2 mos. 18 days.	3rd	13 days	" 5, 0.25 cc. subcut..	Chloroformed in 37 days
				" 6, 0.25 cc. " ..	" " "
				" 7, 0.25 cc. abdomen	Dies in 5 days.
				" 8, 0.25 cc. "	" " 5 "

The second bovine test resulted in the death of the two mice receiving the dose into the abdomen, in 5 days. There were no lesions recognizable. The two mice inoculated subcutaneously were active up to the 37th day, when they were chloroformed. Lesions were not found.

Pigeons. Fowls and pigeons have been used with success in distinguishing avian from mammalian tubercle bacilli. Hence it was thought best to test human and bovine bacilli on pigeons. The cultures used are given below in the table. The bacilli, in the form of bouillon suspensions, were injected into the pleuro-peritoneal cavity, the needle being introduced caudad of the sternum. In one case the injection was made into a wing-vein. The inoculations were in general negative, with the possible exception of that of the swine culture. In this bird there was found hyperæmia of the serosa of the intestines, with an eruption of scattering, minute grayish dots. There were similar opaque points on the air-sac membranes. On the duodenal loop were found some particles of viscid exudate composed exclusively of coarsely granular cells. In this exudate some tubercle bacilli were still recognized by the specific stain. In some of the other pigeons there were a few opacities of the mesenteries and air-sac membranes, with perhaps one or more conspicuous Peyer's patches. Even in refractory animals, lesions such as these might be anticipated where the tubercle bacilli are deposited.

TABLE V.—PIGEONS.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Designation of pigeon.	Dose and mode of injection.	Remarks.
Sput. II	6 mos. 14 d.	9th	11 days	No. 2	1 cc. abdomen...	Chloroformed in 45 days: negative.
" III	3 " 19 "	5th	11 "	" 4	1 cc. " ..	Chloroformed in 57 days: negative.
Bov. II	5 " 26 "	9th	11 "	" 1	1 cc. " ..	Chloroformed in 49 days: negative.
Swine I	1 yr. 19 "	17th	11 "	" 3	1 cc. " ..	Chloroformed in 57 days: minute excrescences on portions of intestine and membranes of air-sacs.
Sput. V.	5 mos.	5th	31 "	" 5	¾ cc. wing-vein..	Chloroformed in 47 days: negative.
Bov. V	8 mos. 14 days	8th	24 "	" 8	1.5 cc. abdomen..	Chloroformed in 49 days: negative.
Horse I	11 mos. 5 d.	11th	12 "	" 7	0.5 cc. "	Chloroformed in 50 days: negative.

Cattle. It is a noteworthy fact that, in spite of the great economic, agricultural and hygienic interests involved in the diffusion of bovine tuberculosis throughout the world, no experiments upon cattle with cultures of the bovine bacillus had been made up to the time of my first experiments, published in the *Transactions of the Association of American Physicians* for 1896. Experiments upon cattle with the human bacillus had been made by several observers. A discussion of these tests I shall defer to the end of this article, because of certain secondary questions, which will be clearer when my tests have been described.

The results of the inoculation of two heifers into the lungs with Bovine culture I and *Nasua* culture have already been published.* It may be stated briefly that the bovine culture produced general tuberculosis, ending fatally in 35 days, while the Nasua (human) culture failed to produce any recognizable lesions.

During the warm season of 1897 inoculations were made with 5 sputum, 1 swine and 4 bovine cultures upon as many cattle. For these 10 animals I am indebted to the Massachusetts Board of Cattle Commissioners, who have shown much interest in the progress of these investigations, because of the need felt by them of more definite information upon the relation of bovine tuberculosis to the human disease. The test was carried out in two series, the first including 4, the second 6 animals. The culture material used was suspended in bouillon, as described, the density of the suspension being about the same as that of a typhoid culture in bouillon 24 hours old. In both series the dose was 2 cc. of such a suspension.

Series I. The 4 animals of this series were inoculated with the following cultures:

Sputum II,	total age	5	months	9	days ;	8th	transfer.
Sputum III	"	2	"	15	"	4th	"
Bovine II,	"	5	"	2	"	5th	"
Swine I,	"	11	"	23	"	16th	"

The swine culture is probably bovine in origin, and it was used on this supposition. Assuming that tubercle bacilli become slowly weakened

* Loc. cit.

in virulence by artificial cultivation, we should endeavor to use cultures as fresh and of as nearly the same age as possible. This theoretical demand cannot be successfully met, because of the many difficulties surrounding such work. Of the 4 cultures used, Sputum II and Bovine II are of nearly the same age, while Sputum III is but half as old as they, and Swine I more than twice as old.

In making the injection, the space between the 6th and the 7th rib was chosen. The needle was inserted about 3 inches above the level of the elbow (olecranon process). It was found subsequently, at the autopsy, that the point chosen was too low, and that in all of the animals the needle, leaving the lungs intact, pierced the diaphragm. Some of the bacilli were discharged into the muscles of the thoracic wall, some into the abdominal cavity and some into the pleural cavity. Bearing this accident in mind, we may now go to a description of the further history of the inoculated cattle. They were all housed in a spacious, well-ventilated barn in large commodious horse stalls. A piece of ground adjoining the barn was enclosed, and in this the animals spent 6 to 7 hours a day for about 4 weeks. Thereafter the animals were separated into two lots, one lot being out several hours in the morning, the other several hours in the afternoon. The two which received the bovine and the swine culture were allowed to run together, similarly the two which received the sputum cultures. It might be claimed that there was in this arrangement a possible danger of transmitting the bacilli from one animal to another and of infecting the ground. There was no evidence of this at the post-mortem examination, and the arrangement was considered safe at the start because it takes some time for the tuberculous tissue to become disintegrated. It was, however, deemed prudent not to keep the animals longer than 2 months, on account of the imperfect isolation.

In apportioning the cultures to the animals, the sputum cultures were injected into the youngest animals, in order that these cultures might have any advantage likely to accrue from difference in age. Two yearlings (without any permanent incisor teeth) received the two sputum cultures, the bovine culture was injected into a heifer about 2½ years old, the swine culture into a heifer about 2 years old.

They were killed and examined at the Brighton (Boston) abattoirs, with the co-operation of the Board of Cattle Commissioners, just two months after the day of the injection. Before injection, these animals had been tested with tuberculin by the Board and found free from tuberculosis. Still, since this agent occasionally allows an animal to escape which contains foci of the disease, attention was directed to this point

at the autopsies. No lesion, however, was found which from its situation and appearance could be referred to any former spontaneous infection.

Let us examine first the effect of Sputum culture II and Bovine culture II, which were of nearly the same age when injected.

The weight of the yearling which had received the sputum culture had risen in the two months from 520 to 580 lbs., that of the heifer from 650 to 710 lbs. There was no continuous fever recognized in either animal, though the temperature was taken twice a day, morning and afternoon. The fluctuations noticed were evidently due to the effect of the sun while the animals were in the enclosure.

The lesions in the yearling were very slight. At the seat of inoculation between the 6th and 7th ribs a mass of tubercles attached to pleura about 1 x ½ inch in dimension, the tubercles composing it partly cheesy, partly firm. Near the cephalic border of ventral lobe of right lung, a subpleural nodule, not yet necrotic, about ⅛ inch in diameter. On abdominal aspect of diaphragm, right side, about 24 isolated tubercles $\frac{1}{12}$-⅛ inch in diameter. A few similar tubercles on the omentum, which is slightly adherent to the large intestine. When adhesion was removed, about 6 or 7 nodules found on caecum $\frac{1}{12}$ inch in diameter. Evidently the injection needle had passed through the diaphragm into abdomen, depositing some fluid there and some in thorax when partly withdrawn.

The lesions in the heifer which had received the bovine culture were quite extensive and were diffused through thorax and abdomen, owing to the penetration of the diaphragm by the needle of the injection syringe:

Thorax. The right pleural cavity shows an abundant eruption of tubercles along the borders of the ribs. Some of the masses formed are characteristically flattish, grape-like and in bulk quite large. One mass measured 8½ x 3 x 1½ inches. Others of similar dimensions were present. On the lateral margin of the right lung a series of loosely attached, flattish neoplasms up to 2 inches in diameter, besides hyperæmic fringes of loose connective tissue. On the convex surface of this lung only a few tubercles. Large patches of tubercles on pericardium and diaphragm.

In the muscular portion of diaphragm, right side, a mass of tuberculous tissue (probably place where needle penetrated) 1½ x 1½ x ½ inch in dimension. The caudal of the series of dorsal mediastinal glands about twice normal size on account of the presence of many small foci, showing in some cases an opaque, yellowish centre.

Abdomen. The omentum densely studded with agglomerations of tubercles covering the greater part of its surface. These masses vary up to ½ inch in thickness. Similar patches on abdominal aspect of diaphragm and on spleen. Fewer patches on gall-bladder and on liver. In one of the portal glands a ¼-inch focus, pale, grayish, permeated with small calcareous spicules.

The examination of sections of the pleural eruptions and of one of the dorsal mediastinal glands showed the typical lesion of bovine tuberculosis, with its foci of epithelioid cells and its numerous giant cells containing a few tubercle bacilli. Any detailed description of these lesions would be simply a repetition of familiar facts.

We have in these cases a wide divergence in the result of inoculation. The human bacillus produced a slight eruption of small, tubercle-like bodies, which did not present even microscopically the characters of true tubercles, while the bovine bacillus produced an exquisite case of pearly disease both in thorax and abdomen, with the formation of large, grape-like masses in the chest.

The youngest sputum culture (III) was injected into a yearling weighing 410 lbs. At the end of two months the weight had risen to 480 lbs. The lesions found are slight:

On the abdominal aspect of diaphragm, right side, a patch of isolated tubercles about 2 inches in diameter, the tubercles themselves about $\frac{1}{6}$ inch in diameter and about $\frac{1}{2}$ inch apart. They are grayish, opaque. At place of inoculation, on serous aspect of ribs, a flattish neoplasm 1 inch in diameter and about $\frac{1}{8}$ inch thick. Other lesions not detected. In this case also the needle evidently entered abdomen through diaphragm. Sections made from the small isolated tubercles on the abdominal surface of the diaphragm show them to be masses of newly forming tissue. They consist in the main of spindle-shaped connective-tissue cells running in parallel and interlacing groups and richly supplied with vessels. No trace of the characteristic tubercle formation or of bacilli, although these bodies are evidently due to the bacilli injected.

The swine culture was injected into a somewhat older animal weighing 620 lbs. After two months the weight was 660 lbs. The autopsy showed the following conditions:

At place of inoculation (right chest wall) a subcutaneous tumor about 2 inches in diameter, made up of a very dense connective-tissue sac, ¼ inch thick, which encloses a pale yellowish semifluid mass (caseous). There are besides this focus, in the same situation, 3 smaller nodules from ½ to ¾ inch in diameter, the largest with caseous centre. On the pleural aspect a similar ½-inch centrally softened focus.

In nearly the centre of the right half of diaphragm and projecting into abdomen for ½ inch is a tumor representing portion of a larger focus in the muscular portion of the diaphragm about 1 inch in diameter. This focus is likewise caseous, diffluent centrally. On all the ribs of right pleural cavity are eruptions of small tubercles, reaching in some instances a diameter of ⅛ inch. Besides the palpable tubercles, there is along one border of each rib a line of vascular fringes of connective tissue. In many of these fringes tubercles are not noticed, although the fringes themselves are evidently a result of the injection.

Along the lateral border of the right lung similar vascular fringes of tissue containing tubercles. These fringes extend dorsad for about 2 inches on ventral and cephalic lobes.

Diffuse eruption of minute tubercles on pleural aspect of diaphragm, right half.

Large caudal mediastinal gland contains large numbers of tubercles, varying in size from mere points to those ⅛ inch in diameter and showing beginning necrosis. Left bronchial gland several times normal size. Ventral mediastinal gland several times normal size and containing several large, centrally caseous foci. One mesenteric gland contains several small necrotic tubercles.

In this case many of the bacilli had been deposited in the fleshy portion of the diaphragm and some under the skin, and had thus been prevented from exerting their greatest power. Sections were made of the subcutaneous foci, the anterior mediastinal and bronchial lymph-glands and of the vascular fringes on the pleural aspect of the diaphragm. The glandular and subcutaneous lesions are characteristically tuberculous, with a greater tendency towards necrosis and disintegration of the tubercles than in the animal inoculated with Bovine culture II. The lesions of the serous membranes are more vascular and show greater tendency toward the formation of connective tissue around the minute tubercles. In general this culture must be considered less potent than the bovine culture. Whether this is due to the more prolonged artificial cultivation or to the species through which the bacillus had passed remains unsettled.

Series II. In this experiment three sputum and three bovine cultures, recently isolated, were used. The bovine cultures were of nearly the same total age. The sputum cultures differed somewhat from them and from one another in this respect.

Sputum	IV,	total age	6	months	10	days,	7th	transfer.
"	V,	"	1	month	19	"	3rd	"
"	VI,	"	1	"	12	"	3rd	"
Bovine	III,	"	4	months	7	"	5th	"
"	IV,	"	4	"	3	"	5th	"
"	V,	"	4	"	3	"	5th	"

The cultures used for the injection were all 9 days old, grown on the same lot of dog's serum. Control inoculations of guinea-pigs and rabbits will be found on tables II and III. The animals at my disposal, unfortunately, varied considerably in age, and in assigning the cultures the advantage was given to the human cultures:

Sputum IV, yearling, 525 lbs., no permanent incisor teeth.
Bovine III, " bull, 645 lbs., no permanent incisor teeth.
Sputum V, cow, 675 lbs., about 4 years old.
Bovine IV, " 850 " " 12 "
Sputum VI, cow, 865 lbs., about 3½ years old.
Bovine V, " 875 " " 6 "

The injection of the cultures was carried out as in Series I, excepting that the point of insertion of the needle was chosen higher up, about 11 inches above the elbow of the animal when in the standing position, between the 7th and 8th ribs. The length of the needles used was about 2 inches. The care of the animals was the same as that bestowed on the preceding lot, excepting that the bovine and the sputum animals were kept separate in the out-door enclosure from the start, the one lot being out in the morning, the other in the afternoon. They were kept two months, with the exception of the young bull, which died 17 days after the inoculation. The four cows of this lot gave at the start altogether about 8 quarts of milk. The secretion was slowly dried up, so that in the sixth week a very little, amounting perhaps to one quart in two days, was removed. The milking was continued chiefly to prevent any udder troubles during the experiment and to maintain normal conditions. The temperature was taken but once a day, at noon.

In comparing the temperature records of these 6 animals it was noticed that the three animals which received the bovine cultures had a high temperature immediately after the inoculation, which lasted until the death of the bull and about three weeks for the remaining two animals. At the same time no such elevation of temperature was recorded for the animals receiving the three sputum cultures. There was but one well-defined rise of temperature in the case of the yearling, from the 13th to the 16th day after inoculation. The other irregularities are probably due to the fact that the temperature was usually taken after these animals had been in the enclosure in the sunshine for several hours. Those with the high temperature were kept much of the time in the cool barn in the morning, which probably depressed the fever curve somewhat. After the period of fever, no other elevations were noted up to the close of the experiment.

The young bull, inoculated with Bovine culture III, showed besides the prompt onset of a high temperature, general and local disturbances about a week after the inoculation. The breathing became rapid, the appetite had partly gone. Emaciation and weakness supervened. He was unable to get up September 11, and died the following night.

The autopsy revealed a severe miliary tuberculosis of both lungs with marked congestion and œdema of the organ. Normal collapse no longer possible. The associated lymph-glands were much enlarged and infiltrated with minute tubercles. Patches of minute tubercles were found on the pleural covering of ribs and on the omentum. Sections from the lungs, dorsal mediastinal glands, pleural neoplasms, liver, spleen and kidneys were examined. In the lungs, mediastinal glands and pleural neoplasms, tubercles were very numerous, in the liver less abundant. In the kidney sections none were seen. In these different situations the tubercles presented nearly the same peculiar features, which distinguished them readily from the tubercles of the spontaneous disease in cattle. To make the differences clearer, a brief description of the lesions in a spontaneously diseased lymph-gland may not be out of place.

A rarefied area within the follicle containing a few nuclei having the characters of endothelioid nuclei and belonging to what are generally known as epithelioid cells, which are imbedded in or whose outlines are fused with a matrix appearing either homogeneous or else very delicately reticulated. In larger foci this rarefied area contains also one or more giant cells whose body is frequently seen merging by prolongations into the reticulated matrix of the tubercle. Among the epithelioid nuclei are isolated nuclei closely resembling those of lymphoid cells. Tubercle bacilli are, as a rule, scarce and situated usually within giant cells. The largest foci are centrally necrotic, that is, they contain a coarse trabecular matrix staining with eosin and picric acid and containing scarcely any nuclei and very little, if any, fragments of nuclear origin. In this mass tubercle bacilli are rarely found. In the lesions of the bull the tubercles have in the first place no giant cells. The new tissue appears to be made up of cells resembling the usual epithelioid cells of tubercle, but much more crowded together and with less definition of the cell outlines. The nuclei are mostly distorted, drawn out, band-like or horse-shoe-like, and twisted into various bizarre forms. Condensation and fragmentation of the nucleus are also seen. In the tubercles in all situations much fibrin is demonstrated by Weigert's stain. This is especially distinct in the liver tubercles. Here the intercellular substance from material hardened in alcohol stains deeply in eosin. In sections subjected to Wei-

gert's fibrin stain, this substance is blue, appearing throughout the tubercle both as a delicate mesh and as very coarse trabeculæ. In material hardened in Zenker's fluid the fibrin stain is less pronounced. In all the tubercles, large numbers of tubercle bacilli are present. These differences are probably due to the rapidity of the process.

On October 27, the remaining five animals were killed at the Brighton abattoirs, with the co-operation and assistance of the Board. The three sputum animals had all gained in weight:

Yearling (Sputum IV) from 525 to 610 lbs.
Cow (Sputum V) from 675 to 750 lbs.
Cow (Sputum VI) from 865 to 960 lbs.

Sputum culture IV. One permanent incisor on the right has appeared since date of inoculation. In utero a fœtus about three months old. At point of inoculation in the subcutaneous tissue a small nodule about ¼ inch in diameter with contents soft, cheesy. Attached to this is another, smaller nodule about ⅛ inch in diameter.

On the right side of chest wall, pleural aspect, there are attached along the six caudal ribs, soft dark red, pendulous masses of newly-formed, highly vascular connective tissue. At point of inoculation, between 7th and 8th ribs, a flattish pediculated mass of tissue of brownish red color. On the 10th rib another mass about ⅜ inch in diameter. The left side of thorax is normal.

Right lung. On the smaller (cephalic and ventral) lobes, newly formed, delicate fringes of hyperæmic connective tissue, which appears also along the free lateral margin as a delicate band about ½ inch broad, as well as on a portion of the convex surface of the lung, occupying exclusively the lines representing the boundaries of the lobules.

In the large caudal lobe, which is similarly beset with the vascular fringes, a tumor, representing the place where needle penetrated the lung tissue, projecting slightly above the convex surface, is found two inches from the caudal tip. This tumor, about ¾ inch in diameter, contained a completely disintegrated mass and about a dozen surrounding foci, $\frac{1}{16}$ to ⅛ inch in diameter, with yellow softened centres. On the margin of this same lobe, in addition to the vascular fringes, are four firm masses of grayish tissue, smooth, flattish, attached by pedicles to the margin of the lobe. The diameters of the largest are ¼ and ½ inch.

On large (caudal) lobe of left lung there is only a very little development of vascular fringes. Imbedded in the same lobe near lateral margin is a uniformly grayish, slightly translucent mass, sharply defined from the enveloping normal lung tissue. Sections of this focus showed a peculiar lymphoid structure of the tumor, in the centre of which a parasite was lodged. Not

having any relation to the inoculation disease, it need not be further discussed.

Attached to the cephalic lobe of the left lung by a pedicle is a small, flattish, smooth mass of new tissue.

The pleural aspect of diaphragm and portions of the pericardium are covered with areas of the highly vascular neoplastic tissue. In some, small nodules can be felt at the free extremity of this tissue.

On the right ventricular surface of the heart four flattish, pediculated masses about as large as split peas are attached.

The various lymph-gland systems, ventral and dorsal mediastinal and bronchial glands, do not show the presence of tubercles or any augmentation in size.

Of this case, sections of the primary lung focus, of two of the pediculated bodies (one from the lung, one from the rib) and of the vascular fringes from the lateral border of the lungs were examined.

The small foci immediately bordering the disintegrated primary focus vary from 0.2 to 2 mm. in diameter. The smallest do not differ essentially from ordinary tubercles, excepting in the scarcity of giant cells. The larger ones are centrally necrotic, with a marked tendency towards encapsulation on the periphery by the condensation of circularly disposed, fusiform, connective-tissue cells. In the smallest tubercles, bacilli are scarce, in the largest they are numerous within the necrotic centre, where they appear as slender, often bent, sometimes fragmented and rather feebly stained rods. In the surrounding epithelioid zone a few well-stained bacilli are present. Passing from the primary place of deposit, the only other lesions are those of the pleura. These occur in two forms, as vascular fringes and as firm, smooth, pediculated bodies, some flattish, like pumpkin seeds in size and outline.

The vascular fringes occur: 1. As mere endothelial tubes; 2. As such enveloped by a layer of wavy fibrillar tissue and covered with a layer of endothelial cells; and 3. As vessels sheathed with a zone of round cells, proliferating endothelial and connective-tissue cells in addition to the fibrillar tissue. Acidophile, polymorphous leucocytes are present here and there throughout the granulation tissue. Mitoses are occasionally seen. After a prolonged search through 7 sections, one minute rarefied area is detected. In it evidently a tubercle bacillus with long axis nearly at right angles to the section.

The vascular fringes from the thoracic aspect of the diaphragm are like those from the lungs. In the former no signs of tubercle formation.

Sections from one of the neoplasms attached to the ribs show two fairly distinct regions, an outer of dense connective tissue and an inner made up of foci of lymphoid elements and a central necrotic core. This latter body was evidently a vascular offshoot of the pleura, which, before necrosis, had attached to or imbedded in it many lymphoid cells, for the necrotic core contains much nuclear matter traceable to such cells. This core is partly envel-

oped by a ring of epitheliod cells with nucleus at the outer free end, like those around a foreign body. Some are multinuclear and resemble giant cells. In the zone immediately outside of this cellular ring are several characteristic giant cells. There are no tubercle bacilli, nor is there any tissue recognizable as epithelioid and identifiable as tubercle.

Sputum culture V. Cow about 4 years old. In utero a fœtus 3 to 4 months old.

Development of vascular fringes along one border of ribs of the right side of thorax as in preceding case, but amount relatively slight. In the intercostal muscles at the point of inoculation a mass of perhaps a dozen small grayish tubercles.

In the large caudal lobe of the right lung, in the same situation as in preceding case, a projecting tumor about 1 inch in diameter. When incised it is found composed of two ½-inch foci of disintegrated, cheesy-viscid matter enclosed in thin, smooth capsules. No surrounding infiltration. Along the margin and on the caudal surface of this lobe, slight development of pendulous vascular tissue and a sessile tubercle about 4 mm. in diameter. On the left caudal lobe only very slight production of vascular tissue.

On the surface of one of the middle dorsal mediastinal glands an aggregation of minute tubercles, the whole about ½ inch in diameter. Imbedded in the cortex of the same gland two minute tubercles.

In this animal the disease of the middle mediastinal gland, although very slight, is shown in sections to be tuberculosis. The lesions are not to be distinguished from those of the spontaneous disease in cattle, excepting in the presence of considerable numbers of tubercle bacilli. The tubercles themselves contain the usual number of giant cells, and the largest ones are centrally necrotic, the necrosed mass being indistinctly fibrillar in character, staining deeply with eosin and devoid of nuclear debris. The bacilli in this mass stain rather feebly when compared with the deep stain of those in the giant cells on the periphery of the tubercle. This lesion will be again referred to in summing up the experiments.

Cow (Sputum culture VI). In utero a fœtus about four months old.

Within the thorax on the right side, between the 7th and 8th ribs, is a small, pediculated, blackish (hæmorrhagic) mass of firm tissue about as large as a pumpkin seed, another on the 10th rib. On most of the ribs behind the 7th are gelatinous-looking pendulous vascular fringes of neoplastic tissue. On the pleural surface of the diaphragm a similar development of vascular tissue and several firm, pediculated masses like those on ribs.

Of the right lung the cephalic lobe shows very slight formation of marginal fringes. In the ventral lobe whose tip is adherent to the pericardial fat, a uniformly grayish, sharply defined focus ¼ inch in diameter.

In the same situation as in the preceding cases the caudal lobe shows a slightly projecting tumor about 1¾ inches in diameter externally. When incised it is found to consist of a smooth-walled sac 1¼ inches in diameter, containing a yellowish curdy mass, together with a little turbid fluid. No surrounding infiltration. On the convex surface of this lobe there is a slight growth of vascular tissue. Near the caudal tip a flattish mass, partly yellowish, partly blackish, attached by loose tissue to the margin of the lobe. It is about 1 inch long and ½ inch thick.

In the abdomen, a flattish, sessile mass of pinkish-gray tissue about ¾ inch in its longest diameter, attached to omentum.

In this animal, sections of the grayish focus in the ventral lobe of the right lung and of the suspicious sessile mass on the omentum demonstrate the fact that both lesions are parasitic in origin and due to the same cause as the lung focus in cow inoculated with Sputum IV. Both foci are made up of spherical agglomerations of lymphoid cells, in the centre of which the parasite is situated.

The largest of the pendulous masses from the lungs was examined microscopically and found to be similar in structure to those already described. It consists chiefly of a connective-tissue stroma of interlacing fibres in which are imbedded many capillaries. Scattered through the inner half of the mass are quite small foci of epithelioid and lymphoid cells, but giant cells are absent. In these foci, a few tubercle bacilli are found. The periphery of this pendulous mass consists of circularly condensed connective tissue, beneath which is a homogeneous, somewhat reticulated substance, staining well with eosin and enclosing a few tubercle bacilli. It is probably a layer of fibrin undergoing hyaline degeneration. Small areas of it are still stainable with Weigert's fibrin stain.

Of the three animals receiving bovine tubercle cultures, the fate of one (young bull No. 71) has already been given. The two other cases remained stationary in weight:

No. 88—original weight 850 lbs.; weight at end of experiment 850 lbs.

No. 63—original weight 875 lbs.; weight at end of experiment 870 lbs.

The autopsy notes are in brief as follows:

No. 63 (Bovine culture V). White cow, spotted with red. Horns sawed off. Probably six years old. Fœtus in utero about two months old.

Thorax. Right lung adherent to chest wall in several places. At point of inoculation, between 7th and 8th ribs, an excrescence of the costal pleura about ¾ inch in diameter, of dense, pearly-looking connective tissue enclosing a disintegrated mass. Numerous masses and aggregations of small tubercles on all ribs. These masses in some cases are several inches in length. The left side of thoracic wall, below the level of the point of inoculation on

the opposite side, is covered with a uniform, pinkish-gray deposit of very minute tubercles. Eruptions of tubercles on pleural surface of diaphragm and on pleural covering of dorsal mediastinal space.

On caudal lobe of right lung a considerable number of tubercular masses, flattish, sessile, from ⅛ to ¾ inch in diameter. Between the cephalo-lateral border of this lobe and the pericardium is a mass of newly formed tissue, dense, in which are imbedded many minute yellow tubercles and masses of pericardial fat. The whole is about as large as a fist. It binds the lung tissue, pericardium and diaphragm together. Many tubercles on the caudal surface of this same lobe. There is no distinct focus in this lobe as a result of the injection, and it is probable that much of the fluid was deposited in the pleural cavity. But palpation reveals throughout both lungs small shot-like bodies in close proximity. On section numerous yellow tubercles from $\frac{1}{32}$ to $\frac{1}{16}$ inch in diameter are found imbedded in the lung tissue of all lobes.

The dorsal mediastinal lymph-glands are all several times their normal dimensions. They contain many coalescing yellow tubercles. The ventral (anterior) mediastinal glands are similarly enlarged and the cut surface shows a uniformly cheesy parenchyma.

Minute grayish points under the capsule of the liver.

In the spleen, all Malpighian bodies converted into tubercles with yellow opaque centre. In left kidney several minute grayish tubercles.

Of this case, sections of the left lung, left costal pleura, the large caudal mediastinal gland and the spleen were examined.

The tubercles from the lungs do not differ from those found in spontaneous tuberculosis. The centre is as a rule necrotic and contains a few tubercle bacilli. Outside of this is a zone of nuclear fragments and entire nuclei, shading off into a peripheral zone of epithelioid and lymphoid cells and cells with spindle-shaped nuclei. Giant cells are present in nearly all the tubercles. The new growth on the left side of the thorax consists essentially of newly formed vascular fringes, in which are imbedded minute tubercles with scarce bacilli and occasional giant cells.

In the caudal mediastinal gland the tubercles vary from a mere giant cell to those 2 mm. in diameter and centrally necrosed. The latter are like those in the lung tissue. The bacilli in some necrotic foci are numerous, in others few in number.

In the spleen the follicles contain epithelioid cell foci, some of which are centrally necrosed and which contain many bacilli.

No. 88 (Bovine culture IV). Red and white cow. Teeth very much worn, probably 12 years old, dehorned. Not pregnant.

No deposit in subcutis at point of inoculation. The eruptions of tubercles on the costal pleura of the right and the left side are in character very much like those of the preceding case (No. 63), but less extensive.

Right lung. Lobes adherent to pericardium. Adhesions readily severed. On convex surface of the caudal lobe of this side a considerable number of flattish sessile tubercles from $\frac{1}{16}$ to $\frac{1}{2}$ inch broad. The caudal aspect of this lobe is similarly beset with them, but in less abundance. Along the margin of this lobe are loosely attached, small, elongated masses of tubercles.

At the same situation in this lobe as in the sputum cases, there is a fluctuating tumor, about two inches in diameter, slightly projecting. It consists of a capsule with nearly smooth walls enclosing a soft caseous mass. It is surrounded by a zone of small necrotic tubercles and with lobules containing numerous minute, grayish foci.

On the cephalic lobe of the right lung are a considerable number of grayish tubercles. Throughout all lobes are scattering tubercles in the lung tissue, some very minute, others larger and opaque, yellowish in color.

The pleura in the dorsal mediastinal space is beset with a large number of small tubercles; similarly the pericardium. The right half of the diaphragm is beset with flattened aggregations of tubercles. Between the ventral lobe of the right lung and the pericardium, and fastening them together, is a mass of newly formed connective tissue and fat enclosing numerous softened foci.

The large dorsal mediastinal lymph gland is enlarged and contains a large number of yellow tubercles. The central portion of the gland is uniformly caseous. In the left bronchial gland a small number of tubercles; in the ventral mediastinal glands a considerable number. Organs of the abdomen appear free from tuberculous changes. Of this case only the tubercles disseminated through the lungs were examined microscopically. For this purpose the left lung was chosen. The tubercles are small, compact up to 1 mm. in diameter. In structure they are indistinguishable from the spontaneous bovine tubercles. The bacilli in them are few in number, rather short, thick and deeply stained.

A summary of the outcome of this last experiment may now be made. The points of difference between the inoculation disease produced by bovine and by human (sputum) bacilli are several.

1. The bovine cases either remained stationary in weight or lost slightly, while the sputum cases gained 75 to 85 pounds. Still, the age of one of the bovine cases may be partly responsible for stationary weight.

2. There was marked fever in the bovine cases for three weeks after the inoculation; practically none in the sputum cases.

3. There were well-marked differences in the lesions produced. In the sputum cases the lesions are nearly the same and consist in:

(*a*) A tumor in the right caudal lobe of the lung, about 1 inch in diameter, projecting somewhat above the surface of the lung. This represents the place where the needle penetrated into the lung tissue and deposited the tubercle bacilli. In each case the contents of this tumor were softened and converted into a curdy mass, enclosed in a thin-walled capsule, smooth internally. The disease was not spreading from this point nor were tubercles visible in the lymph glands of the lungs and thorax, excepting in one gland of No. 39 (Sputum V).

(*b*) The free margin of the right lung and the pleural covering of the ribs on the right side were beset with a new formation of loose vascular fringes or shreds in which in only one case some minute nodules could be felt, and some flattish, pediculated masses, which did not resemble tubercles at all histologically, or which were only abortive forms of the same.

Among the bovine cases we have the following characteristic points to note:

(*a*) Disseminated tuberculosis of the lungs, severest and fatal in No. 71, the youngest; least pronounced in the oldest, No. 88. Associated with this a local disintegrated focus in the lungs of No. 88.

(*b*) Tubercular deposits on lungs, pericardium, diaphragm and the ribs, resembling closely the product of the natural disease in cattle. Extension of the eruption to the costal pleura of the left side.

(*c*) Extensive tuberculosis of all or nearly all the lymph glands of the thorax, including both mediastinal chains.

(*d*) Slight tuberculosis of other organs, spleen, liver, kidney, in two out of three cases.

A summary of the three separate tests on cattle (including the published experiment), in which twelve animals were used, shows that 6 animals were inoculated with human bacilli, 5 with bovine bacilli; 1 animal was inoculated with swine bacilli.

Of the sputum cases, 1 showed no disease; 2 showed very slight lesions; 3 showed only local lesions without dissemination.

Of the bovine cases, 2 died of generalized disease; 2 showed extensive lesions; 1 showed less extensive lesions.

In the swine case the lesions were less extensive than in the bovine cases.

TABLE VI.—CATTLE.

Designation of culture.	Total age of culture.	Number of transfer.	Age of culture used.	Amt. of suspension of bacilli injected.	Designation of animal.	Age, etc.	Original weight in pounds.	Final weight in pounds.	Date of inoculation.	Result.
	mos. days		days							
Bovine I	5 4	3rd	10	4 cc.	No. 284	heifer, 2½ yrs. old, common stock, pregnant.	(?)	(?)	May 4, 1895	Died in thirty-five days of generalized tuberculosis.
Nasua narica	9 9	8th	10	4 cc.	No. 300	heifer, 2½ yrs. old.	(?)	(?)	May 4, 1895	Killed June 27, 1895, no lesions.
Sputum II	5 9	8th	9	2 cc.	No. 2616	yearling.	520	580	May 1, 1897	Killed July 1, 1897, lesions very slight.
Sputum III	2 15	4th	9	2 cc.	No. 2634	yearling.	410	480	May 1, 1897	Killed July 1, 1897, lesions very slight.
Bovine II	5 2	5th	9	2 cc.	No. 2635	heifer, 2½ yrs. old.	650	710	May 1, 1897	Killed July 1, 1897, extensive pearly disease in thorax and abdomen.
Swine I	11 23	16th	13	2 cc.	No. 2672	heifer, 2 years old.	620	660	May 1, 1897	Killed July 1, 1897, well-marked pleural tuberculosis with invasion of lymph glands.
Sputum IV	6 10	7th	9	2 cc.	No. 79	yearling, about 1½ years old.	525	610	Aug. 26, 1897	Killed Oct. 27, 1897, abscess in lungs at point of injection, new vascular tissue on ribs and lungs.
Sputum V	1 19	3rd	9	2 cc.	No. 39	cow, 4 years old.	675	750	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Sputum VI	1 12	3rd	9	2 cc.	No. 76	cow, 3 years old.	865	950	Aug. 26, 1897	Killed Oct. 27, 1897, lesions same as preceding.
Bovine III	4 7	5th	9	2 cc.	No. 71	bull, yearling.	645	575*	Aug. 26, 1897	Died Sept. 12, 1897, disseminated tuberculosis of lungs, liver.
Bovine IV	4 3	5th	9	2 cc.	No. 88	cow, about 12 years old.	850	850	Aug. 26, 1897	Killed Oct. 27, 1897, many minute tubercles in lungs, tuberculous deposits on pleura.
Bovine V	4 3	5th	9	2 cc.	No. 63	cow, about 6 years old.	875	870	Aug. 26, 1897	Killed Oct. 27, 1897, disseminated tuberculosis of lungs and spleen.

* Two days before death.

ON THE SIGNIFICANCE OF VARIETIES AMONG TUBERCLE BACILLI.

The foregoing experiments, while they show unmistakably the close relationship existing among the various cultures studied, nevertheless justify us, if only to guide and stimulate further study, in establishing a distinctively human or sputum and a bovine variety of the tubercle bacillus. It might be better to omit the host designation of such varieties, in order to anticipate assumptions that they are necessarily limited to the host whose name they bear. Still, the convenience of using the host's name is so great that I shall succumb to it. The characters upon which the bovine variety may be based reside, morphologically, in the invariably short, straight form and in the greater resistance of this form to modifying influences of culture-media; biologically, in a greater resistance to artificial cultivation and in a much greater pathogenic activity towards rabbits, guinea-pigs and cattle.

There is proof, furthermore, of the existence of slightly varying characters even within the varieties proposed. Among the bovine forms studied, slight variations in virulence were noticeable. Among the sputum forms, variations in size, in capacity for cultivation, and in pathogenic activity have been observed. The differences between Sputum culture I, on the one hand, and Sputum cultures II to VII, on the other, are quite pronounced. In spite of these variations, mammalian tubercle bacilli may still be considered as forming a fairly compact group when compared with the tubercle bacilli of birds, which are but slightly virulent towards the guinea-pig, so susceptible to the mammalian type.*

In regard to the bovine and the sputum varieties, I have, in a former paper, pointed out certain differences of behavior in the body which may be of considerable significance in explaining their divergent characters. In an organism like the human body, manifesting a certain amount of resistance to the tubercle bacillus, the sputum type multiplies only to a limited extent, unless it can produce dead tissue or gain

*I have, unfortunately, been unable to study avian bacilli in connection with the series of this article, because opportunities for making cultures from fowls were wanting. The study of cultures of unknown or indefinite history I have not regarded as likely to yield trustworthy evidence.

an exit upon mucous membranes secreting pathological products, where it seems to flourish as a saprophyte. Similar conditions are now and then observed in animals inoculated with human bacilli. Immense numbers of bacilli are occasionally found in necrotic foci, but very few in the surrounding zone of living tissue. In the many sections examined recently, the necrotic foci were the ones in which human bacilli were to be found, if detected at all. The bovine type differs from the former in a far less saprophytic growth. In the pathological secretions and in the caseous masses the bacilli are relatively scarce. This difference may be a result of their adaptation to the bovine body, in which cavities of the lungs and catarrh of the air tubes are far less common. In other words, certain differences in the type of reaction tend in the one case to make the human bacillus more saprophytic, the bovine more parasitic.

Coming to the more practical aspect of this subject, we may present, in view of the relationship between the bovine and the sputum variety of the tubercle bacillus, two propositions for discussion:

1. The sputum bacillus is incapable of finding a foothold in the bovine body.

2. The bovine bacillus may pass to the human subject, owing to its higher pathogenic power.

The transmission of human tuberculosis to cattle has been experimentally essayed by Bollinger, Crookshank, Baumgarten, Sidney Martin, the writer and Frothingham.

Bollinger * reported in 1894 an experiment performed in 1879. A calf three months old was inoculated into the peritoneal cavity with fluid from a tuberculous human lung. The calf was killed in 7 months. On the mesentery and the peritoneal covering of the spleen there were found pediculated tumors from the size of a pea to that of a walnut. The microscopic appearance corresponded completely with that of the spontaneous pearly disease of cattle. The retroperitoneal and mesenteric glands were tuberculous. All others were normal. The little drawing which Bollinger published with the article gives one the impression of smooth, roundish tumors similar to those I have described as resulting from the injection of Sputum cultures IV and VI. They have not the

* *Münchener med. Wochenschr.*, 1894, No. 5.

nodulated appearance presented by the spontaneous eruptions or those produced by bovine cultures.

Crookshank * injected sputum containing numerous tubercle bacilli into the peritoneal cavity of a calf. The calf died after a prolonged illness on the 42nd day.

Extensive lesions were discovered at the post-mortem examination. The mesentery was adherent to the rumen; the liver was adherent to the diaphragm. There was extensive tubercular deposit at the seat of inoculation and an abscess the size of a walnut. Extending over the mesentery from this point there were hundreds of wartlike, fleshy new growths, some quite irregular in form, others spherical or button-shaped. There were similar deposits on the under surface of the liver, on the spleen, in the gastro-splenic omentum and on the peritoneal surface of the diaphragm. The spleen was adherent to the rumen, and on dissecting away the adhesions, another abscess was opened. On the under surface of the liver was a third abscess about the size of one's fist, which burrowed in the depth of the liver substance.

On microscopic examination, extremely minute tubercles were found disseminated throughout the lungs and liver. Tubercle bacilli were found in these organs and in the peritoneal deposits. The pus from the liver abscess contained streptococci. The calf died of pyæmia, a result to be anticipated if sputum be employed for inoculation, but sufficient time had elapsed for pronounced local infection leading to acute miliary tuberculosis.

I quote this experiment more fully so that it may discredit itself as a means of deciding whether sputum can produce general miliary tuberculosis in cattle. We know as yet too little of the influence of concurrent infections to place any reliance upon the outcome of this experiment as a demonstration of the transmissibility of human bacilli to cattle.

Baumgarten † briefly mentions an experiment in which human bacilli in cultures had but little local effect after intraocular inoculation upon a calf. Material from a bovine pearly nodule produced typical local and general miliary tuberculosis in another calf.

Sidney Martin ‡ fed one kilogram of tuberculous material from a cow to 4 calves at one meal with their food. Within 4 weeks tubercles were found in the Peyer's patches, mesenteric and bronchial glands and in the lungs of one animal. After 8 weeks, another calf presented the same lesions plus tuberculosis of the posterior mediastinal and the cer-

* *Trans. Pathol. Soc.*, London, 1891, 332.

† *Jahresbericht* for 1891, footnote, 666.

‡ Report of the Royal Commission on Tuberculosis, 1895. Appendix, p. 18.

vical glands. The third calf, killed after 12 weeks, showed ulceration of the small intestine and cæcum and lesions of the pleura, besides those mentioned above. The fourth calf, killed after 37 weeks, had tuberculosis of the pleura in addition to that of the various lymph glands already mentioned.

Four calves were fed at one meal with 70 cc. of sputum containing a large number of tubercle bacilli. Three calves, killed after 4, 8 and 12 weeks respectively, had 53, 63 and 13 nodules respectively in the small intestines, chiefly in Peyer's patches. In the fourth calf, killed after 33 weeks, no lesions were found.

Two calves received at one feeding 440 cc. of sputum, containing a large number of tubercle bacilli. In one calf, killed in 8 weeks, 13 tuberculous nodules were found in the intestine. The mesenteric glands were also affected. The other calf, killed in 19 weeks, was found without any lesions.

The author infers from these experiments that "in the case of tuberculous sputum we are dealing with material which is less infective to calves than bovine tuberculous material."

Frothingham * carried out some experiments of a similar character for the Mass. Cattle Commission. A culture of human bacilli was used, isolated from the liver of a child and about 1 year old. Two calves, 3 months and 3 weeks old respectively, received suspensions of the culture into the peritoneal cavity. In both only slight local nodules were produced, some resembling spontaneous tubercle, others tending towards granulation tissue. Two calves, 3 weeks and 2 months old respectively, were inoculated into the trachea. In one case the large local abscess in the muscles of the neck indicated a deposit there of much of the material destined for the lungs. In the liver and lungs a small number of minute tubercles, devoid of tubercle bacilli, were found. In the other calf lesions were absent. Thus, in spite of the immature age of these animals, the tubercle bacillus may be said to have had but a trifling local effect on them. The tests on guinea-pigs indicate a very attenuated culture. Three additional calves inoculated subcutaneously with sputum containing many tubercle bacilli showed lesions equally slight.

The defect of most of these tests is the absence of parallel experiments with bovine tubercle bacilli. Inasmuch as we do not yet know anything concerning the relative resistance of cattle to tuber-

* Report of Mass. Cattle Commission for 1897. See also *Zeitschr. f. Thiermed.*, 1897, 330.

culosis at different periods of life, nor the relative effect of cultivation on the bacilli and of different modes of inoculation, such parallel experiments are quite essential. Of these few experiments, Bollinger's is the only one which might be regarded as favoring the susceptibility of very young cattle to human bacilli. Even here the disease penetrated only to the mesenteric and retroperitoneal lymph glands.

A summary of the inoculation experiments made by the writer upon cattle have already been given. Here we need simply to report the most salient features. These are—

1. The purely local, restricted character of the lesions in cattle due to sputum bacilli. Lesions were not found beyond the place of deposit or in the lymphatic glands to which these are tributary, excepting in one animal (Sputum V culture). In this case we find a multiple tubercular focus measuring *in toto* about 1 to 1.5 cm. in one of the dorsal mediastinal glands. This is the only affected gland found in the 5 cases. Considering the fact that the bacillus was one of the more attenuated, that on the other hand the animal was fully 4 years old, I am strongly inclined to look upon this isolated instance of lymph gland infection as a latent infection with bovine bacilli which was probably favored by the depressing effects of the injection into a slight activity. I take this ground because of its isolated occurrence and the very typical bovine character of the lesions. It is not unusual for such a slight lesion to be encountered in animals which have failed to react towards tuberculin, for experimenters agree upon a margin of error of 5 to 10 per cent, the lesions found in such cases being usually quite insignificant. Had the culture used not shown a virulence not only not above but slightly below the average virulence of the other sputum bacilli, as tested on a rabbit and on guinea-pigs, the above inference could not be justly drawn.

2. The atypical tissue changes induced by the sputum bacilli which tended chiefly towards granulation tissue. Tubercle formation recognizable as such was uncommon. Besides the lymphatic focus cited above as of doubtful origin, tubercles approaching nearest the bovine type were encountered in the primary lung focus in the youngest animal (Sputum IV).

Some writers, including Bollinger and Baumgarten, are inclined to regard the pediculated masses produced on serous membranes by the inoculation of sputum or of cultures (Sputum IV, VI) as something specific and as deciding positively the transmissibility of human bacilli to cattle. Leaving aside the abortive, atypical, microscopic structure of the pediculated masses produced in the writer's series, and assuming that the inoculation of young calves may furnish lesions of a more typical character, we may present anatomical and histological facts which go far to show that there is nothing characteristic or peculiar about them or about pearly disease in general.

On the omentum and the pleura of cattle not tuberculous there may exist in many cases patches of delicate filamentous fringes, probably in all cases the result of some local inflammation. These fringes are microscopic in size and only visible to the naked eye when they are present in large numbers and congested. My attention was first called to them in the study of Texas fever. Owing to the congestion of the whole portal system, these fringes, massed together, appeared as red patches on the omentum. The microscope showed them to consist of a capillary encased in a sheath of delicate fibrillar tissue and the latter covered by the endothelium of the peritoneum. Recently I have demonstrated the same fringes in slightly raised, reddened patches of the costal pleura in otherwise healthy cattle. Here they are quite inconspicuous, but may be demonstrated by seizing the surface of the membrane with delicate forceps. Invisible threads hold the latter near the membrane. In the fresh condition their structure is like that of the fringes on the omentum. In some I was unable to demonstrate a capillary. In all cases they consist of the wavy fibrillar tissue devoid of nuclei, but containing at times a few granule cells, recognized as acidophile leucocytes in stained sections. In sections, these fringes could be traced from their attachment to the serosa. The fibrillar tissue is directly continuous with the subserous layer of like tissue and the endothelial layer of the pleura is continuous over these fringes. The immediate union of fringes containing a capillary with the pleura was not demonstrated in the limited number of sections examined. Besides these of the simplest structure, others are seen in an active state of proliferation and infiltrated with lymphoid and acidophile leucocytes.

These fringes or filaments, probably the result of traumatic and other inflammation, form an excellent place of attachment for the injected tubercle bacilli. Here processes of epithelioid and giant-cell formation go

on rapidly when bovine bacilli are present, whereas the sputum bacilli tend to lead only to granulation tissue, with slight abortive tubercle formation. These new formations, subject to pressure and friction, may undergo hæmorrhage, anæmic necrosis due to twisting of the pedicle, and subsequent increase of the connective tissue stroma around the dead fringe. In this way those peculiar smooth nodes probably arise which are briefly described under the cases inoculated with Sputum cultures IV and VI. It is, furthermore, evident from the inoculation experiments that, among other causes, the tubercle bacillus itself is one inducing the formation of granulation tissue. The highly vascular, velvety layers of granulation tissue carpeting the costal pleura in most of the cattle inoculated is a sufficient proof of the existence of this tendency. The pearly disease may thus be a product of at least two factors, the presence of this tissue as a result of former proliferation, and the inherent tendency of the serous membranes of cattle to respond by the formation of these vascular fringes to tubercles situated under the pleura and to bacilli discharged subsequently from such tubercles. But still other factors may be concerned.

That a certain retarded movement of the disease process may produce pearly disease in other animals has been answered in the affirmative for rabbits by Troje and Tangl.* These authors inoculated subcutaneously bacilli from cultures and material from the tuberculous lungs of rabbits which had been intimately mixed with iodoform and kept in the dark in contact with it for one or two weeks. In two rabbits, from two separate experiments, chloroformed respectively 7 and 9 months after inoculation, pediculated tubercles of the serous membranes were found. Those not containing angular concrements, and therefore sectionable, were peculiar in the possession of a very large number of very large giant cells.

These observations I am able to confirm by one case, the rabbit inoculated with the culture of Sputum VI. This rabbit was chloroformed 3½ months after the intravenous injection of tubercle bacilli. Its weight had risen from 1305 to 2061 grms. The notes on this case are briefly as follows:

Organs apparently unaffected (brain and cord not examined), excepting the liver, kidney and lungs. The liver contains a small number of 1 to 2 mm. centrally caseous foci. The kidneys show a considerable number of grayish opaque 1 mm. tubercles. The lungs collapse well. They are everywhere beset with translucent bodies, seen with some difficulty, owing to their glassy

* *Deutsche med. Wochenschrift*, 1892, 191.

appearance. Some have a minute, point-like, opaque centre. In the small cephalic lobes there are larger confluent patches, especially on the margins, of a peculiar grayish-translucent appearance. Sections of hardened tissue show the distinctly epithelioid character of the tubercular changes, with very few giant cells. Necrosis only in the largest foci. Tubercle bacilli fairly abundant in the necrosed centres, elsewhere scarce. From the marginal tubercles finger-like prolongations, precisely like those of the bovine pleura, project into the pleural cavity. They consist mainly of rapidly proliferating connective-tissue cells imbedded in a very delicately fibrillar meshwork. The basal portion of the fringe is partly covered with endothelium continuous with that of the pleura and contains a number of capillaries in transverse section. Similar vascular fringes appear on the opposite margin of the same section.

It is not improbable that if this animal had been allowed to live as long as those of Troje and Tangl, tubercle bacilli would have found their way into these fringes and produced the so-called pearly disease. This rabbit was allowed to live longer than any other of the sputum series. We may thus assume that neoplasms of the pleura in rabbits may be looked for after the third month. These observations go far to show that the eruption of tubercles on the pleura is not so much an expression of peculiarity on the part of the tubercle bacillus as it is an expression of a certain grade of virulence of the bacillus in an organism possessing a certain grade of resistance to it. To these conditions may possibly be added a pronounced tendency in certain animals towards production of granulation tissue of a certain type on serous membranes.

Troje * has also described a rare case of pearly disease in man, affecting the pleura. Here, possibly, the previous existence of outgrowths like the fringes on the serous membranes of cattle may have been responsible for the peculiar localization of the tubercle bacillus.

Putting all the facts obtained by experiments upon cattle together, it would seem as though the sputum bacillus cannot gain lodgment in cattle through the ordinary channels. These avenues, well provided with protective mechanisms, receive the bacilli probably one at a time. However closely the sputum and the bovine bacillus may be related, it seems as if under ordinary circumstances the former would fall an easy prey to destruction. This inference will gain in weight if we bear in mind that the far more potent bovine bacillus produces in at

* Loc. cit.

least 50 per cent of the spontaneously infected cattle a purely local disease, which probably would remain so if the animal were surrounded with favorable conditions.

The second and most important proposition, the transmission of bovine bacilli to the human subject, has been much discussed in recent years, without, however, bringing us any nearer to definite knowledge.

A recent compilation of cases of presumable transmission from cattle to man, both through wounds of the skin and through the digestive tract in milk, by Ravenel,* gives perhaps the most condensed account up to date. To these may be added a most interesting case reported by Coppez.† A girl 17 years old had a wound on the palmar aspect of the third finger between the 2nd and 3rd phalanx, which became infected with tubercle bacilli during milking. The original lesions gave rise, within 6 months, to over 35 subcutaneous abscesses situated in different parts of the body. There were two on the right hand, four on the elbow, two on the shoulder-blade, several on the right cheek, two on the left palm, one on the back of the neck, a dozen on the buttocks and thighs, four on the left leg, three on the sole of one foot and one on the big toe. Most of these were curetted, and iodoform applied 6 months after the beginning of the disease. Subsequently more appeared, in all from 60 to 66. The author describes the appearance of these foci as follows:

At a certain point, always in the neighborhood of a pre-existing focus, a thickening of the subcutaneous tissue appeared, associated with increased heat of the skin and pain on pressure. The heat soon disappeared, and a fluctuating livid tumor opened itself and discharged pus and later a yellowish serum. These various abscesses healed slowly, and within a year all had disappeared. At no time could any visceral lesions be recognized, but one eye became involved. The nature of the affection was demonstrated by inserting some pus from an abscess into the eye of a rabbit. There was but slight reaction at first. Subsequently the whole eye became diseased, the lymph nodes of the neck being greatly enlarged. After several months death ensued.‡

* *Journal of Compar. Medicine and Vet. Archives*, December, 1897.

† Un cas de tuberculose cutanée et oculaire sans manifestations viscérales, *Rev. gén. d'ophthalm.*, xv (1896), 433.

‡ In an article written just before the discovery of the tubercle bacillus, Creighton (Bovine tuberculosis in man, *Journ. Anat. and Physiology*, xv (1880), 1-177) describes 12 cases, following one another quite closely in the same hospital, which he considers to be afflicted with the bovine disease. This type of tuberculosis was characterized by eruptions on the serous mem-

If bovine bacilli may invade the human body without let or hindrance, we have not only food infection through milk and milk products to guard against, but also the inhalation disease to which men are exposed in stables containing tuberculous cattle. What proportion of tuberculous subjects may derive their infection from these sources we do not know. Now that we have established some fairly pronounced differences between bovine and sputum bacilli, the whole discussion might be cut short by the suggestion that the time has come to stop citing old and doubtful cases and to go to work to study with care the tubercle bacilli from cases of supposed animal origin, so that some experimental, trustworthy basis may be formed upon which to found statistics. While this is in truth what will have to be done and is the goal which has been aimed at from the outset in this tedious work, it will take much time and persistent attention to collect evidence of this kind. In the meantime the relation of bovine to human tuberculosis must be somehow defined before a fairly helpless and frightened public. It seems to me that, accepting the clinical evidence on hand, bovine tuberculosis may be transmitted to children when the body is overpowered by large numbers of bacilli, as in udder tuberculosis, or when certain unknown favorable conditions exist.* To prevent this from occurring, a rigid, periodic dairy inspection and the removal of all suspicious udder affections and all emaciated animals is as much as public health authorities can at present demand. Any measures beyond these belong to agriculture, with which the public health has no business to meddle, without endangering the chances of

branes, large, grayish-white medullary tumors in the lungs, frequently assuming the form of a wedge, and by certain microscopic peculiarities. These cases may have been due to some other organism. Quite recently Askanazy (Ueber tumorartiges Auftreten der Tuberkulose, *Zeitschr. f. klin. Med.*, xxxii (1897), 360), besides giving a good bibliography, cites 2 cases in full in which the peculiar type of disease leads him to suggest their relation to pearly disease in cattle. These articles simply impress upon the reader that the true interpretation of such cases will remain obscure until the etiological agent has been studied. The variable character of the tissue reaction to the tubercle bacillus, according to the species, deprives the pathological study, taken alone, of any decisive value.

* Probably much the same as in glanders. The number of glandered horses in certain places is out of all proportion to the cases in men who are continually handling and cleaning them.

gaining authority to enforce its own necessary measures. If the evidence gained by pathology in the future should reveal a greater danger than is here assumed, the scientific basis of such evidence will, I think, force all additional measures needed.

But for the student of etiology the problem does not end in the differentiation of varieties. It reaches out much farther than this and involves some puzzling questions. The most important one bears on the possible changes which the tubercle bacillus may undergo during its prolonged sojourn in the human body. I have already referred to one phase of this question in mentioning the saprophytic growth of the sputum bacillus in the affected lungs and necrotic tissue, as contrasted with its slight multiplication in living tissue and with the generally slight multiplication of the bovine bacillus in the tissue of cattle. This question is a very complicated one, and nothing is easier than to reason in a circle about it, because of the entire absence of data. The first hypothesis to be considered is that which assumes the conversion of the bovine bacillus into the sputum bacillus in the human body. This hypothesis would deny us all possibility of utilizing different characters of tubercle bacilli in tracing their source. If the bovine variety may enter into the digestive tract as such, and after a more or less prolonged interval emerge from the secondarily affected lungs as the sputum variety, we may as well give up all further study of the tubercle bacillus. The accumulated evidence of bacteriology is opposed to this view. Nevertheless, it has been unwittingly assumed by Klebs, in considering intestinal infection with the bovine bacillus, as a common cause of tuberculosis.* As a support of the view that tubercle bacilli change but very slowly, I may again mention the inoculation test upon a rabbit of Bovine culture I. About three years after the first test, the second test resulted in only a very slight prolongation of the disease. The first rabbit succumbed in 17, the second in 22 days. It was thus after 3 years of artificial growth still far above sputum bacilli in pathogenic power.

The question of phthisis as secondary to infection by way of the digestive organs is, however, one needing more attention, for experi-

* Die kausale Behandlung der Tuberkulose, 1894, p. 34.

mental results in this direction are quite suggestive. In all mammals the lungs are evidently the most favored place of tubercle bacilli, and wherever the latter may be deposited, they sooner or later, unless the disease is checked, reach that organ, where the process spreads more rapidly than elsewhere. This march from the place of infection is not infrequently partially concealed by reparative processes. A recent case in point is that of a guinea-pig inoculated with a culture of Sputum VI:

Aug. 29, 1897. Male guinea-pig, weighs 420 grams. Receives subcutaneously in the flank 0.5 cc. of a clouded suspension of tubercle bacilli (Sputum VI) 9 days old.

Nov. 8. Breathing now labored; has been active since inoculation.

Nov. 11. Respiration labored and almost convulsive; hence chloroformed; weight 431 grams.

No ulcer at point of inoculation.

Kneefold glands of this side as large as peas, yellowish in color, consisting of a thin-walled sac and homogeneous contents, resembling thick cream. Kneefold glands of opposite side moderately enlarged, but without necrotic changes. The pelvic, renal, retrogastric and mesenteric glands enlarged slightly, but without necroses. Spleen small, shows grayish specks disseminated through it. Liver and kidneys appear normal. Two small tubercles on left testicle.

Lungs do not collapse. They feel firm everywhere. On section most of the lung tissue is consolidated, of a grayish-white color. No fluid expressible from air tubes. In sections of the lungs, the infiltration consists chiefly of epithelioid cells with smaller, more deeply stained nuclei intermingled or in patches. No necrosis. The liver was, through an oversight, not preserved. The spleen shows in each follicle a rarefied area occupied by epithelioid cells and a few tubercle bacilli. No necrosis.

In this susceptible animal the only lesions threatening life or even conspicuous were those in the lungs. Those in the spleen were slight, possibly stationary. The disintegrated superficial glands might have discharged outwardly later on and healed. This is but one of a number of cases I have observed in the past 10 years in which there was progression in the lungs with almost complete destruction of the

organ and retrogression elsewhere, showing itself in the liver by shrinking and furrowing of the organ.*

With the two facts before us that tubercle bacilli gravitate, as it were, towards the lungs in all the susceptible mammals, and that they may conceal their movements in the body quite effectually, we must regard infection through the digestive tract as a source of phthisis at least deserving more attention. The only question to interest us here is the relation of the bovine bacillus to this process. Only much painstaking work will enable us to learn whether the human body can produce such a great modification of the bovine bacillus or not.

Another more acceptable hypothesis concerning changes in the tubercle bacillus would assume a slow, continuous modification which in the past has developed the sputum variety on the one hand and the bovine variety on the other. The future may enable us to establish not only other varieties linked to certain animal species, but also varieties which produce slightly different forms of disease in man himself.† The saprophytic life of the sputum bacillus in phthisis may be the cause of a very slow downward descent of its invasive power, which in the course of one or more generations may become perceptible both to the bacteriologist and to the clinician, and express itself to the statistician in the gradually falling curve of tuberculosis, as shown by the vital statistics of the past 40 years.‡

The outcome of such slow changes would lead to the establishment of numerous minor varieties because of the great opportunity which tubercle bacilli have in our congested, abnormal city life of selecting their variously predisposed victims. Thus the more attenuated varieties may find their invasion obstructed in the more vigorous, but not

* This generalization has been recently emphasized by Spengler (*Ztschr. f. Hygiene*, xxvi (1897), 321) as follows: The retarded course of an inoculation tuberculosis expresses itself without exception in a (greater) prominence of the lung disease with simultaneous retardation of the liver and spleen affection.

† Recently I have been able to study cultures of tubercle bacilli from a rapidly progressive tuberculous gland of the neck in an adult. These bacilli are slightly less virulent than the sputum bacilli described.

‡ S. W. Abbott. The Vital Statistics of Massachusetts. Rep. of the State Board of Health for 1896, p. 787-8.

in children or certain predisposed individuals. It is needless for me to illustrate these possibilities still farther. They have their parallels in nature all about us. Only one practical suggestion may be made, in view of the probable discovery of minor varieties of human bacilli. Tuberculous patients should not be indiscriminately exposed to other tuberculous patients simply because both are affected with a disease which bears the same name.*

A more exhaustive experimental study of the tubercle bacillus away from the many variable factors acting upon the human body, which will lead to a better standardizing of virulence or invasive power, may help these speculations to find some substantial basis. That much is still to be learned concerning the etiology is evident from the want of unanimity as regards the channels of infection. The views of Koch and his followers of the transmission of the infection in dust and spray would tend to favor the hypothesis of gradual attenuation and of the production of minor varieties. Baumgarten's hypothesis of the transmission of the tubercle bacillus from parent to child before birth implies the transmission of tubercle bacilli either in a continual struggle with living tissue or else remaining in situations where the bacilli cannot multiply, and hence where a tendency to loss of invasive power by saprophytic growth is impossible. The occasional entrance of bovine bacilli into the human body might open the way for the introduction of a virus of a higher level, provided opportunity for subsequent transmission be afforded.

* A beginning in this comparative study of different clinical types of tuberculosis has been made by Auclair (*Arch. de méd. exp.* (1897), ix, 1124). This article, which came to my notice after the completion of this manuscript, describes 4 cultures, one from a rapidly progressive tuberculosis of a gland of the neck, one from a slow phthisis, one from an acute meningitis and one from true scrofula. On guinea-pigs the intra-abdominal inoculation of these cultures had nearly the same effect. Hence the author did not hesitate to regard them as identical in pathogenic power, in opposition to the views of Arloing, who considers the bacilli of phthisis and those of the surgical forms of tuberculosis of different virulence. Auclair did not inoculate rabbits, in spite of the fact that Arloing regarded them as the best means of differentiating the bacilli of the different forms of tuberculosis. My own results show that if the intra-abdominal inoculation of guinea-pigs is the only test made, practically no differences worth considering would appear between bovine and sputum bacilli, as regards pathogenic power.

If in this brief summary I have presented nothing but problems to be solved and doubts to be entertained, I feel quite confident that the comparative study of tubercle bacilli will lead to some definite understanding on certain important questions, and eventually to more light on the whole subject of tuberculosis from the preventive as well as the therapeutic side.

The main questions proposing themselves to the investigator are:

1. The study of tubercle bacilli from different types of tuberculosis to determine their relation to the sputum bacillus and the bovine bacillus as regards virulence.

2. The study of the bacilli in primary intestinal disease and in all tubercular disease in children in which the source of infection is assumed to be outside of the family and possibly in the milk.

In attacking these problems the investigator should either keep strictly to the methods suggested in this article, if he wishes to use its results as a basis, or else, in all cases in which the bovine bacillus or one of abnormal virulence is suspected, he should study fresh cultures of the sputum bacillus side by side with the other.