through the regenerated nerve. Here, in my opinion, lies the fallacy, for some of these cases might have recovered, if fixed in this position for a sufficient period, without transplantation. In support of this, Wittek of Gratz, knowing my views upon this subject, sent me a report of one of his cases. It was sent on from the neurological department with a report that the reaction of degeneration was present in muscles supplied by the external popliteal nerve. Relying on this report, he performed muscle transplantation, and the result was successful. A year later the patient came back with an extreme valgus, the internal malleolus touching the floor. On examination the muscles previously pronounced to be hopelessly paralysed were now acting strongly, while the previously strong groups were paralysed from overstretching. There was nothing for it but to reverse his former operation and put his transplanted muscle back where he found it. His remark was, "What a brilliant result to record if I had only done nerve transplantation!" Many of us have had similar experiences in cases where we have been hasty in transplanting muscles.

Although the results of neuroplasty in poliomyelitis leave much to be desired, there is no occasion to abandon hope. We may learn in time the lessons taught by failure, and, what is infinitely more important, take courage from success. A failure depresses, but a success is eloquent of possibilities, and that is just the encouragement we want. Failure should merely set us thinking, and, however critical our mind, it should always remain an open one.

FURTHER INVESTIGATIONS OF THE TYPE OF TUBERCLE BACILLI OCCURRING IN THE SPUTUM OF PHTHISICAL PERSONS.

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THIS research is a continuation of the work on the subject which I carried out for the Royal Commission on Tuberculosis.¹

In my sputum investigations for the Commission I obtained and tested cultures from the sputum of 29 persons suffering from pulmonary tuberculosis. The cultures isolated from 27 of these 29 patients were pure cultures of human tubercle bacilli, while those from the remaining 2 were pure cultures of bovine tubercle bacilli. The experiments with the sputum of the latter two patients were repeated—in one case thrice, in the other once—and it was shown conclusively that tubercle bacilli of standard bovine type only were being expectorated with the sputum of these two patients. These two cases are the first, and are so far the only ones recorded, in which pulmonary tuberculosis in human beings can be definitely referred to bovine tubercle bacilli.

The only other cases of pulmonary tuberculosis investigated in England, in which sputum was the "original" material, are 2 by Cobbett² (for the Royal Commission) and 23 by Bulloch.³ From these cases tubercle bacilli of the human type alone were isolated.

Thus, out of 54 phthisical patients in England, 2 were discovered to be discharging with the sputum none but the bovine type of tubercle bacillus, and 52 none but the human type of bacillus.

This proportion of cases expectorating bovine tubercle bacilli is remarkably high when regarded in the light of results obtained in similar researches in other countries. The last paper on the subject of which I am aware is by Lindemann.⁴ This author gives a list of all the sputum investigations carried out down to the year 1912. The total number of cases investigated in countries other than England was 736. In 733 of these cases the sputum was demonstrated to contain only the human tubercle bacillus; in 2 cases there was experimental evidence that the cultures isolated were mixtures of human and bovine tubercle bacilli, while in the remaining one it was not certain whether the bovine tubercle bacilli, which were undoubtedly present in the sputum, were present alone or in company with human tubercle bacilli.

Further investigations of the type of tubercle bacilli occurring in the sputum of consumptives in England were obviously needed to ascertain whether or not bovine tubercle bacilli are so frequently the cause of consumption in this country as the above cited English figures would suggest. The opportunity of renewing my investigations of the subject was afforded me by my appointment to one of the scholarships of the Grocers' Company. My further inquiry began in August, 1911, and has been pursued intermittently up to the present time, as my work on other varieties of human tuberculosis permitted.

So far the type of tubercle bacillus has been determined in the sputum of 148 additional persons suffering from pulmonary tuberculosis. The sputum samples from 105 of these persons were obtained from the Brompton Hospital for Consumption by kind permission of Dr. Perkins. For the remaining 43 cases I am indebted to Sir Robert Philip, who kindly allowed me to collect samples from the Victoria Hospital for Consumption, Edinburgh.

The object of the inquiry has been not only to discover cases of pulmonary tuberculosis which were expectorating bovine tubercle bacilli with the sputum, but also, and especially, to study the clinical course of such cases, and, if they ended fatally and a necropsy was obtained, to make careful investigation of the distribution, character, and bacteriology of the tuberculous lesions.

The latter investigation is of especial importance, since nothing is at present known in regard to the anatomical pathological picture of tuberculous disease in a person who has during life been coughing up bovine tubercle bacilli. The five persons referred to above, in whose sputum bovine tubercle bacilli were demonstrated, all died, but no *post-mortem* examination was made of any of them.

Another important matter of which there is as yet no actual evidence is the transmission of bovine tubercle bacilli from man to man. Examination and observation of the relatives and intimates of a person who is expectorating bovine tubercle bacilli should show whether or not this transmission readily occurs.

BACTERIOLOGICAL INVESTIGATION.

The sputum used for investigation was that which was coughed up in the early morning. Special precautions were taken to avoid contamination of the sputum with food that might contain tubercle bacilli of bovine origin. During the twenty-four hours previous to collecting the sample the patient was not allowed any butter, nor any milk which had not been boiled. The samples were sent to me in sterile jars, in batches of from nine to fifteen. An attempt was made in each case to obtain a culture direct from the sputum by means of antiformin. When the direct cultures were unsuccessful, or appeared likely to fail, cultures were made from guinea-pigs whenever these had been available.

Every sputum was not inoculated into the guinea-pig. When no guinea-pig had been employed and direct cultures proved negative, a fresh sample of sputum was sent for. In a few instances it was not possible to obtain a fresh sample, the patients having left hospital much improved, and not expectorating. In a few other instances direct cultures and guinea-pig inoculations were both negative. The sputum in these cases had not shown any tubercle bacilli on careful microscopical examination.

All the strains isolated have been tested as to their cultural characters on serum, and on one or more of the differential glycerin media (serum, agar, potato or broth), the medium most frequently employed being glycerin potato. The virulence of the tubercle bacilli present in a majority of the sputum specimens has been tested on the rabbit. In one case calves and goats were used as well.

Methods of Cultivating Tubercle Bacilli direct from Sputum by means of Antiformin.

After trial of various proportions of sputum, antiformin, and diluent (physiological salt solution) the method employed for some little time has been as follows: 1 c.cm. of thick muco-purulent sputum was pipetted into a sterile centrifuge tube (when the sputum was mucoid or contained much saliva, 2 or 3 c.cm. were taken). To this salt solution to make about 9.5 c.cm., and then 0.5 c.cm. antiformin were added; the sputum was then stirred up, and after five, ten, or fifteen minutes, according to the speed with

which the sputum dissolved, the mixture was centrifuged.* The deposit was washed with salt solution, recentrifuged, and then sown upon four tubes of the egg medium.

This washing was later on found to be unnecessary, the amount of unreduced antiformin lingering in the deposit being insufficient, apparently, to interfere with the growth of the tubercle bacilli. A further simplification of the technique of cultivating tubercle bacilli from sputum was suggested by the following observation. It happened in some of the above experiments that the sputum was not completely dissolved when the antiformin-sputum mixture was centrifuged; that, instead of a granular deposit, a deposit of transparent mucinous sputum was obtained. This was nevertheless used to sow cultures, and was found to give a higher proportion of successful results than the granular deposit.

The success which attended the use of such incompletely dissolved sputum suggested the possibility of cultivating tubercle bacilli directly from a mixture of sputum and dilute antiformin after they had been in contact in a test tube for a sufficient length of time to kill all contaminating organisms. Some preliminary experiments have shown that it is possible to obtain cultures in this way.

Equal parts—0.25 c.cm. is sufficient—of a 10 per cent. dilution of antiformin and muco-purulent sputum were pipetted into a small test tube and thoroughly mixed. After the lapse of from five to thirty minutes tubes of the egg medium were sown each with a loopful or two of the transparent brownish mucinous mixture, and were incubated in the usual way. By means of this simple method I have obtained pure cultures of tubercle bacilli from nine different sputa.

Treatment for five minutes with 5 per cent. antiformin was not always successful in destroying contaminating organisms, though these succumbed to longer exposure. In one experiment it was shown that a mixture, which after five minutes' treatment grew contaminating organisms, was sterile of these organisms after treatment for half an hour, the tubes then sown producing eventually numerous colonies of tubercle bacilli. On the other hand, in two other experiments 2 per cent. antiformin acting for five minutes was found sufficient to kill all organisms other than tubercle bacilli. My experience of the method is as yet limited, but the number of positive results obtained prove it to be a practicable method and a great simplification over the somewhat tedious centrifuging process.

The simple method should, perhaps, be restricted to sputa in which the tubercle bacilli are numerous. When tubercle bacilli can be demonstrated microscopically in small numbers only or not at all, the centrifuging method by which the tubercle bacilli present are concentrated into small bulk is more likely to succeed.

Further experiments are being made to determine the most suitable percentages of sputum and antiformin in the mixture, and the shortest period of exposure necessary for given percentages of antiformin to destroy organisms other than tubercle bacilli usually present in sputum.

The presence of free antiformin in material used for sowing cultures has been proved by control experiments to be not detrimental to the growth of tubercle bacilli. In each of two experiments 10 per cent. antiformin was added to an emulsion made from a guinea-pig's tuberculous gland (the tubercle bacilli in one experiment were of the human type, in the other they were of the bovine type) and culture tubes were sown immediately with the mixture. There was not apparently any difference in the number of colonies produced by the mixture and by untreated emulsion diluted to the same extent.

The antiformin mixtures in the above two experiments were further used after varying periods up to twenty-four hours to sow cultures. There was, as time progressed, gradual diminution in the number of tubercle bacilli capable of giving rise to colonies, but there were a few tubercle bacilli still alive at the end of twenty-four hours.

In the cultural experiments with centrifuged deposits of sputum after treatment with 5 per cent. antiformin there has been great variation in the number of colonies produced from different sputa, though all were rich in

tubercle bacilli and all received practically identical treatment. Sometimes the surfaces of all the egg tubes sown were thickly beset with colonies; sometimes the colonies were sparsely distributed; on many occasions only a single colony has grown upon one of the four egg tubes sown.

I have not been able to decide to what this variation is due. There are four suppositions which occur to me as possible explanations: (1) That tubercle bacilli lose vitality in sputum which is kept. (2) That many of the tubercle bacilli in some sputa are dead when expectorated. (3) That owing to differences in physical quality of sputum antiformin may be more destructive to tubercle bacilli in some sputa than in others. (4) That some strains of tubercle bacilli are less resistant to antiformin than others.

There seems no doubt that the numbers of tubercle bacilli which are capable of giving rise to colonies after antiformin treatment undergo diminution in some sputum samples when they are kept. In several instances I have repeated an experiment with a particular sputum, in some after an interval of twenty-four hours, in others after an interval of three days, using precisely the same technique as in the first experiment. In a large proportion of cases the number of colonies produced was markedly less in the second experiment than in the first; often the second series of cultures remained sterile. The diminution in number of colonies was more marked in the series in which the interval was three days than in the one in which the interval was only twenty-four hours.

As all my sputum specimens were several hours old (the Edinburgh ones being at least thirty-six hours old) when first dealt with, my failures to grow tubercle bacilli direct may have been due to changes which go on in sputum subsequent to expectoration and which cause the death of the tubercle bacilli or possibly diminish their capacity to resist the action of antiformin.

How far the production of a small number of colonies, or no colonies at all, from sputa rich in tubercle bacilli is explainable on any of the other three hypotheses it is impossible for me to say. These are questions which can be decided only by investigators who are in a position to use perfectly fresh sputum and to make repeated observations on the sputum of the same patient.

GENERAL RESULTS OF THE INQUIRY.

The number of strains of tubercle bacilli which have been obtained in culture from the sputum of 148 persons suffering from pulmonary tuberculosis is 186. From 115 of the sputum samples (115 individual patients) the cultures were isolated direct by means of antiformin. From 63 sputum specimens (63 patients), of which 30 had yielded cultures direct, cultures were obtained through the guinea-pig.

The cultures derived from 147 of the patients were eugonic, while those (9 strains) obtained from several samples of sputum from the remaining patient were dysgonic.

I will first summarize briefly the results of the experiments with the eugonic strains, and then give a detailed account of the experiments performed in connexion with the case which yielded dysgonic cultures.

The Cases which Yielded Eugonic Cultures.

The 147 patients in this group varied in age from 10 to 49 years; 6 were under 15 years of age; 27 were over 30 years old; the rest were from 15 to 30 years of age. About three-fifths of the patients were males, and two-fifths females. The 177 strains isolated from the sputa of these 147 patients produced on all the test media employed the luxuriant growths which are characteristic of the human type of tubercle bacillus.

The virulence of the tubercle bacilli present in 80 of these sputa has been tested on the rabbit, in 27 instances by the subcutaneous inoculation of sputum, in 51 instances by the subcutaneous or intravenous inoculation of culture, and in 2 instances both by the subcutaneous and intravenous inoculation of culture.

Of 27 rabbits inoculated subcutaneously with sputum from 27 patients, 2 died in 55 and 58 days, and showed a local caseous abscess (in one the nearest glands were also affected) and slight tuberculosis of the lungs. The rest of the rabbits were killed after periods varying from 84 to 104 days. In 2 of these 25 rabbits a local lesion only was

* To enable the centrifuging to be accomplished without exposing the fluid to the air the wool plug is made with a muslin sheath, the margins of which can be turned down over the lip of the tube and secured there with a rubber band.

found; in one there was a local lesion and slight tuberculosis of the nearest glands. All the rest showed a local caseous abscess and tuberculosis of the lungs, the disease in the latter organs being slight in 22 rabbits, and moderately severe in 2 rabbits; the nearest glands were affected in 6, and the kidneys in 5 of these 21 rabbits.

Of 43 rabbits inoculated subcutaneously with 10 to 50 mg. of culture from 43 patients, 3 died in 41 to 53 days, and showed a local caseous abscess and slight tuberculosis of the lungs, the nearest glands of one of the rabbits also being tuberculous. The remaining 40 rabbits were killed (a few died, but not of tuberculosis) in periods varying from 86 to 160 days. One of these showed a local lesion only. All the rest showed, besides a local caseous abscess, some amount of dissemination; the lungs were tuberculous in each of the 39 rabbits, slightly in 33, and moderately severely in 6; the kidneys were slightly tuberculous in 11 rabbits; the nearest glands were tuberculous in 25, normal in 14 rabbits; in one rabbit there was tuberculosis of the mamma. Rabbits to the number of 15 were inoculated intravenously with culture from 12 patients, the dose for each rabbit being 0.01 mg. The pathogenic effects produced by these cultures exhibited the range of variation which is usual after the intravenous inoculation of human tubercle bacilli.

There was no indication from the pathogenic effects produced in any of the 85 rabbits, whether inoculated with sputum or with culture, of the presence in any of the sputa of tubercle bacilli of higher virulence than typical human tubercle bacilli.

With regard to the eugonic cultures from the remaining 67 cases, I decided not to test these on rabbits. The cultures were quite clearly "human" in their cultural characteristics, and all that remains unknown in respect to them is whether any of them contained a slight admixture of bovine tubercle bacilli.

The Case which Yielded Dysgonic Cultures.

On account of the possibility of contamination of sputum with bovine tubercle bacilli ingested with food, a single demonstration of this type of tubercle bacillus in the sputum of a consumptive person is not sufficient to prove that the pulmonary disease in the patient is produced by bovine tubercle bacilli.

There must be repeated demonstration of the bovine tubercle bacilli in the sputum, collected with rigid precautions to avoid accidental contamination.

In the present case, as soon as it was recognized that the cultures isolated from the first sputum specimen were dysgonic, a request was made for a fresh sample. Subsequently three more samples were obtained, and altogether five samples of sputum from this case have been investigated within a period of about four and a half months. The results of the experiments in connexion with each sample are given in detail below.

Calves and goats as well as rabbits have been used in this case for the virulence tests for the purpose of adducing decisive evidence as to the nature of the tubercle bacilli discharged by the patient.

Clinical History.

The patient was a girl, aged 16 years 7 months, a distemper by occupation. She was admitted to the Royal Victoria Hospital for Consumption, Edinburgh, on May 24th, 1913, and had been ill about eight weeks. She was a breast-fed child and had had no serious illnesses during childhood. She was in the habit of drinking unboiled milk until very shortly before admission. On admission she was anxious-looking and pale, and complained of "bloodlessness." There were slight cough, slight expectoration (half a drachm per diem), haemoptysis and pain in the chest. The appetite was good; teeth fair, some molars missing. The glands in the neck were palpable, but were not enlarged to such an extent as to be noticeable by the patient. Her height was 5 ft.; weight 5 st. 7½ lb.; temperature 102.7° F.

Physical Signs.

On the right side of the sternum there was a protrusion of the chest wall extending from the second interspace down to the fifth costal cartilage and laterally from the edge of the sternum to the junction of rib and cartilage. There was dullness at both apices, most marked on the right side posteriorly, and slight dullness over the right lower lobe; a few crepitations were heard posteriorly on the right side and over the left lower lobe; over the right apex vocal resonance was increased, and there was whispered pectoriloquy in front and cavernous breathing behind; the wall of the chest over the left lower lobe was hyper-resonant.

Tubercle bacilli in moderate numbers were found in the sputum on May 26th, June 18th, and July 9th, 1913.

For the clinical notes of the case I am indebted to Dr. Power, formerly resident medical officer, Royal Victoria Hospital, Edinburgh.

Bacteriological Investigation of Sputum: First Investigation.

The first sample of sputum was received on June 19th, 1913. The sputum was thin, purulent, and contained numerous tubercle bacilli, mostly short and well stained, a few only being of medium length and slightly beaded. On the day of receipt cultures were sown with the centrifuged deposit of 1 c.cm. of the sputum after treatment for ten minutes with 5 per cent. antiformin. On the day following a small quantity of the sputum was inoculated intraperitoneally into a guinea-pig. The guinea-pig was killed thirty days after inoculation and cultures were sown from its tuberculous spleen and sternal glands.

The direct cultures were positive, scattered filmy transparent colonies becoming visible in the third week. The tuberculous tissues of the guinea-pig produced on plain egg extremely numerous minute transparent colonies, while on one tube of glycerin egg no colonies and on another only scattered colonies were obtained. On glycerin media (potato, agar, broth, and serum) both the direct strain and a strain from the guinea-pig were dysgonic and identical in cultural characteristics with tubercle bacilli of bovine type.

The virulence of the virus has been tested on (shorthorn) calves and rabbits. Seven rabbits were inoculated with the direct strain and two with the guinea-pig strain.

Calves.

A bull calf weighing 50.5 kilos was inoculated subcutaneously on August 26th, 1913, with 50 mg. of the direct strain of culture (Rabbits 687 to 691 were inoculated at the same time, see below). The calf developed a large local lesion and adjacent gland, and was ill for a little while and then recovered. The animal was killed 252 days after inoculation. Weight at death = 115 kilos.

Necropsy.—Caseous and softened local lesion and caseous and calcareous prescapular gland, each being the size of a hen's egg. Spleen contained moderate number caseous gritty encapsuled nodules; liver and lungs showed sparsely scattered minute tubercles. Thoracic, portal, and coeliac glands contained moderate number retrogressive calcareous or caseous and softened nodules. Many other lymphatic glands contained from 1 to 4 caseous gritty nodules; there was no *Perlsucht* disease.

The slight disease produced in this calf is probably attributable to high individual resistance, since all other animals inoculated with the same culture developed typical acute disease.

On October 13th, 1913, the calf experiment was repeated. A bull calf weighing 44.5 kilos was inoculated subcutaneously with 50 mg. of the direct culture. The animal developed a very large local lesion and enlargement of adjacent glands, became ill in the fourth week, and was killed when very ill on November 29th, 1913, forty-seven days after inoculation. The necropsy showed a large caseo-necrotic local lesion, large caseous prescapular and cervical glands, and severe general military tuberculosis of all the organs and glands identical in every respect with the general tuberculosis produced after similar interval of time by tubercle bacilli of bovine origin.

Rabbits.

The experiments on rabbits are tabulated.

No. of Rabbit.	Dose in mg.	Method of Inoculation.	Duration of Life.	Result.
652	0.1	Intravenous	D. 18 days	General military tuberculosis.
653	12.0	Subcutaneous	D. 33 "	General military tuberculosis.
687	0.1	Intravenous	D. 24 "	General military tuberculosis.
688	0.01	"	D. 17 "	General military tuberculosis.
689	10.0	Subcutaneous	D. 19 "	Caseous lesion; nearest glands caseous; early general military tuberculosis; death in each case premature.
690	10.0	"	D. 26 "	
691	20.0	"	D. 19 "	
785	10.0	"	D. 62 "	General tuberculosis.
786	10.0	"	D. 44 "	General tuberculosis.

Second Investigation.

The second specimen of sputum was received on August 21st, 1913. The sputum was mucoid, and did not amount to more than about 1 c.cm.; 2 c.cm. salt solution were added, and the sputum was emulsified by shaking; 1 c.cm. of this diluted sputum was drawn into a syringe and made up to 8 c.cm. This dilute emulsion of sputum was used to inoculate four rabbits, two receiving each 1 c.cm. intravenously and two receiving each 2 c.cm. subcutaneously, and two guinea-pigs (dose 1 c.cm. each intraperitoneally). A smear preparation of the first dilution of sputum showed numerous tubercle bacilli, mainly

short and uniformly stained. The two rabbits inoculated intravenously died of general miliary tuberculosis in twenty-four and thirty-two days respectively. The two rabbits inoculated subcutaneously died of general tuberculosis in fifty-nine and sixty-four days respectively. In all four rabbits the disease was identical with that which is produced by bovine tubercle bacilli.

One of the guinea-pigs died in thirty days, the other was killed after thirty-seven days; both showed severe general tuberculosis. Direct cultures remained sterile. Cultures were raised from each of the two guinea-pigs, and both strains were found to be identical in cultural characteristics with those isolated from the first sputum specimen.

The virulence of one guinea-pig strain was tested on a goat and two rabbits. An adult nanny-goat, weight 36 kilos, was subcutaneously inoculated on October 18th, 1913, with 10 mg. of culture. The goat died forty days after inoculation. The *post-mortem* examination showed, besides a local ulcer and caseous prescapular gland, severe miliary tuberculosis of the lungs and slight miliary tuberculosis of the abdominal organs, and some abdominal lymphatic glands. The two rabbits were inoculated subcutaneously each with 10 mg. They died in thirty and thirty-three days respectively of general miliary tuberculosis identical with that set up by typical bovine tubercle bacilli.

Third Investigation.

The third specimen of sputum was received on September 26th, 1913. The sputum was muco-purulent, and microscopically showed numerous blue-stained organisms and only a moderate number of short tubercle bacilli; 2 c.cm. of the sputum were vigorously shaken with 18 c.cm. salt solution, a homogeneous, slightly milky emulsion being produced. With this emulsion the following inoculations were made, the doses being expressed in terms of sputum:

	Dose.	Method of Inoculation.	Duration of Life.	Result.
Rabbit 749	... 0.02 c.cm.	Intravenous	D. 42 days	General miliary tuberculosis.
Rabbit 750	... 0.04 c.cm.	"	D. 35 "	General miliary tuberculosis.
Rabbit 751	... 0.02 c.cm.	Subcutaneous	D. 103 "	General tuberculosis identical with that set up by bacilli of bovine tuberculosis.
Rabbit 752	... 0.02 c.cm.	"	D. 54 "	
Guinea-pig 1,091	0.02 c.cm.	Intraperitoneal	K. 35 "	General tuberculosis of moderate severity.
Guinea-pig 1,092	0.04 c.cm.	"	K. 18 "	

Calf 49, weight 49.5 kilos, was inoculated intravenously with 0.5 c.cm. sputum and died thirty-seven days after inoculation. The necropsy showed the lungs extensively consolidated and closely beset with caseous miliary tubercles, the thoracic glands much enlarged with the cortices advanced in caseation, the spleen beset with tubercles, slight miliary tuberculosis of organs and of the lymphatic glands. Tubercle bacilli were numerous in smear preparations.

Goat 34. A young animal, about 7 or 8 months old, weight 14 kilos, was inoculated subcutaneously with 0.25 c.cm. sputum. The animal died forty-three days after inoculation, and showed *post-mortem* a small caseous local lesion, caseation of the prescapular gland, the lungs consolidated and closely beset with miliary caseous tubercles, the thoracic glands enlarged and irregularly caseated, slight miliary tuberculosis of spleen, kidneys, and some lymphatic glands. Cultures were obtained from each of the two guinea-pigs inoculated with sputum. Both strains were identical in cultural characters with those from the first two sputum specimens. The strain from guinea-pig 1,092 was inoculated subcutaneously into three rabbits, each receiving 10 mg. of culture. Two of the rabbits died prematurely. The third was killed when ill thirty-two days after inoculation, and showed general tuberculosis.

Fourth Investigation.

The sputum for this investigation was received on October 25th, 1913. Owing to an error in my instructions the specimen was collected without the usual precautions being observed. On this account neither the sputum nor the culture obtained from it were used for animal experiment. A smear preparation of the sputum showed moderately numerous tubercle bacilli, mostly short, and occurring here and there in small clumps. Cultures were obtained direct from the sputum, and these were identical in cultural characteristics with those derived from the first three specimens. There was no sign of the presence of human tubercle bacilli.

Fifth Investigation.

The last specimen of sputum was received on November 3rd, 1913. The sputum was muco-purulent and microscopically contained moderately numerous tubercle bacilli, all short and frequently occurring in groups. Cultures were obtained direct by means of 5 per cent. antiformin; moderately numerous filmy transparent colonies appeared on all the tubes.

Small quantities of the sputum were inoculated into a rabbit subcutaneously and into a guinea-pig intraperitoneally. The rabbit died of general tuberculosis, typically "bovine," in sixty-five days. The guinea-pig died of general tuberculosis in eighteen days and cultures were isolated from its lesions.

The direct strain and the guinea-pig strains were all dysgonic and identical in cultural characters with those obtained from the first four specimens.

The direct strain was inoculated into two rabbits subcutaneously, the dose in each case being 10 mg. of culture. They died of general miliary tuberculosis in thirty and thirty-two days respectively.

Subsequent History.

On January 11th, 1914, a request was made for another specimen, but I was informed that there had been no sputum for a fortnight, and that the girl was considerably improved in health. She was discharged towards the end of January.

[While this report was in the printer's hands I learnt that the girl had died suddenly at her home and that, unhappily, no *post-mortem* examination was made.]

The results of the experiments recorded above prove conclusively that the tubercle bacilli which this girl was expectorating during the period of 137 days covered by the investigation were wholly dysgonic and were identical in cultural characters and virulence with typical bovine tubercle bacilli.

There can be little doubt but that the bovine tubercle bacilli came from the lungs of the girl. She presented the clinical signs of pulmonary tuberculosis and there was no sign of tuberculosis in any other part of the respiratory tract or in the pharynx.

Had the case been one of pulmonary tuberculosis due to the human tubercle bacillus, in which the sputa had become secondarily contaminated by bovine tubercle bacilli, the cultures isolated would have exhibited the cultural characters of the human tubercle bacillus, and the presence of bovine tubercle bacilli would have been demonstrated only by animal experiment, as in Kossel's case (see below).

But though tubercle bacilli were numerous in the sputum there was no evidence culturally of the presence of human tubercle bacilli in any of the nine strains isolated in the five investigations. Each of the strains was undoubtedly a pure culture of bovine tubercle bacilli.

The discovery of this third case of pulmonary tuberculosis, in which the bovine type of tubercle bacillus exclusively was being expectorated in the sputum, is the direct outcome of the researches of Fraser and Mitchell.

These observers reported that infections with bovine tubercle bacilli were very prevalent among the child population of Edinburgh and district, about 60 per cent. of cases of bone and joint tuberculosis (Fraser) and 90 per cent. of cases of cervical gland tuberculosis (Mitchell) being ascribed to infection with bovine tubercle bacilli.

This reported prevalence of bovine infections in Edinburgh children led me to extend my sputum investigations to Edinburgh. For it seemed to me probable that my quest of bovine tubercle bacilli in sputum would be more quickly successful among a community where bovine tubercle bacilli had frequent opportunity of entering the human body than among one where such opportunity was more restricted.

This expectation was realized, but the number of cases investigated is as yet too small to show whether bovine tubercle bacilli are more frequently associated with phthisis pulmonalis in Edinburgh than in London.

The present position is that bovine tubercle bacilli have been demonstrated in one out of 43 Edinburgh cases and in two out of 159 London cases.

The investigation of the tubercle bacillus occurring in the sputum of phthisical persons in Edinburgh is being continued.

I am indebted to Professor G. Sims Woodhead for the facilities he has given me for carrying out the bacteriological investigations at the Field Laboratories of the University of Cambridge. The cost of the animals used in the research has been defrayed by the Grocers' Company.

SUMMARY OF SPUTUM INVESTIGATIONS IN THIS COUNTRY AND ABROAD.

It will be of interest and value for comparative purposes to append a summary of the results of the sputum investigations in this country and other countries, and to give also a brief account of the five previously recorded cases where bovine tubercle bacilli were demonstrated in the sputum.

The following table is a continuation in condensed form of the table compiled by Lindemann (*loc. cit.*). This table was begun by Dieterlen⁵ and continued first by

Moellers⁶ and then by Lindemann. I have condensed it by adding together the cases, 88 in number, investigated by the first fifteen authors (for whose names see Dieterlen's and Lindemann's papers).

Name of Investigator.	No. of Cases.	Classification of Cultures.		
		Human.	Bovine.	Mixed.
Various authors	88*	87	—	1†
Dieterlen	50	50	—	—
Kitasato	152	152	—	—
Park and Krumwiede	296	26	—	—
Moellers	51	51	—	—
Jancsó and Elfer	5	5	—	—
Commission Final Report ...	29	27	2	—
Kossel	46	45	—	1
Bulloch	23	23	—	—
Weber and Dieterlen	9	9	—	—
Lindemann	41	40	—	1
Own recent cases (London) ...	105	105	—	—
" " (Edinburgh) ...	43	42	1	—
	938	932	3	3

* One incompletely investigated case of Mohler and Washburn excluded.

† The de Jong-Stuurman case.

The 88 cases of "various authors" include 2 from the British Commission's Second Interim Report, which were omitted from the Final Report because in one case only bovine animals and monkeys and no rabbits had been tested with the culture in unmeasured dose, and in the other no special precautions had been observed in the collection of the sputum.

I have included them because in the first of the two cases the experiments on bovine animals and monkeys appear to me to show quite clearly that the culture obtained from the sputum was a pure culture of human tubercle bacilli and because in the second the neglect of precautions had not led to the contamination of the sputum with bovine tubercle bacilli.

In the appendix to the Final Report there is another case which was omitted by the Commissioners from their Report because no precautions had been observed in the collection of the sputum. As the culture from this sputum was eugonic and showed no evidence of the presence of bovine tubercle bacilli, I have included this case also in the table. This is the reason why the number given in the table opposite "Commission Final Report" is 29 instead of the generally quoted official figure 28.

BRIEF ACCOUNT OF THE FIVE CASES IN WHICH BOVINE TUBERCLE BACILLI WERE DEMONSTRATED.

The de Jong-Stuurman Case.

The patient was a peasant woman, aged 27. A glycerin serum culture from the sputum produced fatal general tuberculosis in three calves (the subcutaneous method of inoculation was employed for one calf, the intravenous and intrapulmonary for the others). No rabbits were tested. The culture is stated to have grown luxuriantly. Only one sample of sputum was investigated.

The data in this case are insufficient for definite conclusions. The results of the experiments on calves are evidence that bovine tubercle bacilli were present in the culture, but the fact that the culture grew luxuriantly points strongly to the presence in it of human tubercle bacilli also.

Kossel's Case.⁷

The patient was a female letter carrier, aged 27 years. Sputum for investigation was collected twice—once on May 30th, 1910, and again on June 4th, 1910. From the first sample of sputum a culture of human type was isolated through the guinea-pig and a culture of bovine type was obtained through the rabbit. From the second sample of sputum a pure culture of human tubercle bacilli was isolated direct; a mixed culture was obtained from a guinea-pig which had been inoculated with the sputum, and a bovine culture was isolated from a rabbit which had been inoculated with a particle of the spleen of the guinea-pig.

The evidence is conclusive that the sputum of this woman contained bovine as well as human tubercle bacilli.

Lindemann's Case.

The patient was a musician, male, aged 20 years. Three samples of sputum were investigated. A culture isolated from each sample through the guinea-pig grew on broth like the human tubercle bacillus. Each of the cultures produced in rabbits slight tuberculosis typical of infections with human

tubercle bacilli, except that one rabbit in each series showed a tubercle or two in the kidneys.

As in Lindemann's experience renal lesions were rarities in rabbits inoculated with human tubercle bacilli, he isolated through the guinea-pig a culture from the kidney tubercles of one of the rabbits (second sputum), and attempted, but failed, to secure a culture from the kidney tubercles of another (first sputum). The rabbit culture obtained was dysgonic and highly virulent for calves and rabbits. A virulent culture was also derived from a guinea-pig which had been inoculated with the second sputum sample. Primary serum cultures from the spleen of this guinea-pig had been preserved and were used when they were eleven months old to inoculate guinea-pigs for the purpose of recovering the culture. The culture was secured, but not until after two passages through the guinea-pig.

Though no experimental evidence of the presence of bovine tubercle bacilli in sputum samples A and C is adduced, Lindemann thinks that the occurrence of tubercles in the kidneys of rabbits inoculated with cultures of these sputa is best explained on the assumption that small numbers of bovine tubercle bacilli were present in these samples also.

But small renal lesions, such as occurred in Lindemann's rabbits, are not sufficient evidence of the presence of bovine tubercle bacilli in material inoculated.

In my experience small tubercles in the kidneys are by no means rare in rabbits subcutaneously (or intravenously) inoculated with pure cultures of human tubercle bacilli. Lindemann's case should be regarded, therefore, as representing, at most, a single finding of bovine tubercle bacilli in sputum.

British Commission's First Case.⁸

The patient was a butcher's assistant, aged 21 years. He had had pneumonia and pleurisy ten months before admission to hospital and had suffered from cough ever since. When admitted there was consolidation of upper part of left lower lobe. Four specimens of his sputum were investigated. All contained numerous short tubercle bacilli. Cultures from the first sample, isolated through the guinea-pig, exhibited the cultural characters and the high virulence for the calf, rabbit, and guinea-pig of a typical bovine tubercle bacillus.

The other three samples were collected at intervals of 76, 117, and 118 days respectively after the first. Cultures were obtained from each sample through the guinea-pig, and from the last two direct by means of antiformin. All the strains were identical in cultural characters with those first isolated and proved highly virulent for rabbits, the direct culture from the third specimen producing rapidly fatal tuberculosis in two calves inoculated subcutaneously.

British Commission's Second Case.⁸

The patient was a bricklayer, aged 31 years. He suffered from pneumonia when 20 years old and from pleurisy four months before admission. When admitted there were signs of consolidation of both upper lobes. Two specimens of sputum were investigated; the second specimen was collected 118 days after the first. Both samples contained a moderate number of tubercle bacilli. The cultures isolated from the two specimens through the guinea-pig and from one specimen directly were very dysgonic and exhibited for calves, rabbits, and guinea-pigs the high virulence of typical bovine tubercle bacilli.

REFERENCES.

- ¹ Final Report, Part II, Appendix, vol. i.
- ² Second Interim Report, Part II, Appendix, vol. ii.
- ³ Horace Dobell Lecture, Royal College of Physicians, London, 1911.
- ⁴ *Tuberkulose-Arbeiten a. d. Kais. Gesundheitsh.*, Heft 10, 1910.
- ⁵ *Ibid.*, Heft 12, 1912.
- ⁶ *Veröffentlichungen der R. Koch. Stiftung zur Bekämpfung der Tuberkulose*, Heft 1, 1911.
- ⁷ *Deut. med. Woch.*, 1911, No. 43.
- ⁸ Final Report, Part II, Appendix, vol. i, pp. 8-10 and 149-152.

FOUR conventions of nurses will hold their triennial sessions in San Francisco during the Panama-Pacific International Exposition. The four organizations of nurses which will visit the exposition are, according to the *New York Medical Journal*, the International Association of Nurses, representing fifteen foreign nations, including England, Ireland, Germany, France, Belgium, Italy, and others, with Australia, China, and Cuba, of which Dr. Helen Parker Griswell is president; the American Nurses' Association, with 22,000 members, of which Miss Genevieve Cook, of San Francisco, is president; the National League of Nurse Education, numbering 12,000 members, chiefly educators and superintendents of training institutes; the Organization of Public Health Nurses, with an equal number of members, whose labours have to do particularly with public health, tuberculosis, settlement work, social service, and the like. These are distinct from the Red Cross, which also will hold a congress at about the same time. The California State Nurses' Association will act the part of hostesses to the foreign contingent. The attendance from European countries will, it is said, be very large. In addition to the congresses, these organizations are uniting in the preparation of a comprehensive exhibit of modern hospital appliances, besides an historical exhibit, a model mortuary, and demonstration wards. These exhibits, with the demonstration hospital, will be maintained during the ten months of the exposition.