

Dock (Geo.)

THE EXAMINATION OF SPUTUM
IN THE DIAGNOSIS OF TU-
BERCULOSIS.

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A LECTURE AND DEMONSTRATION IN THE
TEXAS MEDICAL COLLEGE, DELIVERED
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Gentlemen:—Before proceeding to the demonstration, let me recall to your mind certain facts tending to show the importance of the subject. Consumption is the most widely spread and most fatal of all diseases. About one-seventh of all deaths are due to it. In fact, were diseases feared in proportion to the number of their victims, phthisis would far outrank, in that respect, the most dreaded of the exotic plagues which at times ravage the earth. According to the statistics of the Imperial Board of Health of Berlin, more people die in five years in that city from phthisis than have succumbed to cholera in its fifteen epidemics in the same place. In Calcutta, the cradle of that disease, the proportion of death is about half that from consumption in the cities of the temperate zone. The epoch-making researches of Koch, published in 1882, have confirmed the belief that phthisis as an almost invariable rule is tubercular, and proved that the cause is as invariably a micro-organism, the bacillus tuberculosis. It is one of its characteristics that this anatomical product, tubercle, tends to break down and to ulcerate through adjacent membranes. In this process the cause of the disease, the bacillus, is liberated along with the broken down products of its activity. Soon after the announcement of Koch, an immense number of investigations showed that in all cases of phthisis, from a time when ordinary signs are not yet present, so long as there was expectoration of disease-products, the bacteria could be found in the sputa. The manner in which this occurs can be clearly learned by an inspection of the specimen under the microscope. This is from a lung showing peri-bronchial tubercles. In the field you see the cross section of a small bronchiole. At one side is a group of miliary tubercles forming a nodule the

size of a hemp seed. This has broken through the wall of the bronchiole; and you see the granular mass of tuberculous matter lying in its lumen. That it is of recent occurrence can be learned by noting the cylinder cells of the bronchial mucous membrane lying in the cavity. No doubt the rupture occurred shortly before death; another cough would have caused the expulsion of the mass. So early in the disease do such liberations of bacilli take place that they can be found before there is enough alteration of structure in the lungs to give the usual signs. Let me illustrate by citing a case. P. H., aet. 34 years, of superb physique and good history, acquired a bronchitis as the result of exposure in July, 1889. He recovered from it in two or three weeks, but did not recover his usual weight and at the end of four weeks had lost 12-14 lbs. His appetite and digestion were good. Percussion of his chest was normal. Careful auscultation revealed nothing but an expiration probably prolonged over the right apex. He coughed hardly once a day and sputum could be obtained with difficulty. Finally a small quantity, his total for a day, not more in bulk than a five cent piece, was secured. Examination for bacilli revealed them in small numbers. The examinations were repeated weekly. No change occurred in physical examination; the quantity of sputum remained constant; there was about one coughing spell a day. The patient gradually lost weight, however, and about six weeks after discovery of the bacilli a few fine rales could be heard over the right apex at the end of expiration. The patient was then advised to go on a ranch in the western part of the state, where his condition has improved slightly and his chances of recovery are no doubt very good.

Taught by numerous like instances



careful physicians have come to make similar examinations in every case where expectoration is a symptom and cannot be attributed to some non-tubercular cause. Indeed, even cases which seem clear in other ways can often have much light thrown on them by a sputum examination. No doubt some of you remember the man in St. Mary's Infirmary whose case seemed one of fetid bronchitis. The routine examination revealed a sputum containing elastic fibres and tubercle bacilli.

In examining sputum for any purpose we should go about it in an orderly and methodical manner, otherwise valuable information may be missed. Sputum is a very composite product. Under ordinary circumstances it is made up of secretions and products of disease of the lungs, bronchia, trachea, and larynx; particles of food and secretions of the mouth, pharynx and their various glands; and of the secretions of the nose, which may run back to mix with the rest. For our purpose many of these are unnecessary, and some even impede our search. Tuberculosis of the nose or mouth being rare, and permitting discovery without a sputum examination, we should endeavor to obtain only the sputum from below the epiglottis. To do this we must instruct the patient to avoid aspirating back his pharyngeal and nasal secretions, to exclude food particles by cleanliness, and to avoid giving us saliva alone. Bacilli can often be found in saliva, but a negative result would of course be valueless. We furnish the patient with a clean, wide-mouthed vial and tell him when he feels an impulse to cough to empty his mouth of saliva, to eject the sputum coughed up into the vial and to add nothing in the way of preservative. Such additions make the preliminary examination of sputum difficult, and are not necessary for the preservation of

the bacilli. The purer the sputum the greater the ease and certainty of our examination. Having the sputum, our first object is to make a careful macroscopic inspection of it. This is best done by spreading it out on a clean pane of glass and examining it over a dark surface. Under certain circumstances it is advisable to have a white back-ground too. In this naked eye examination we determine the form, color, and consistence of the sputum. Most frequently it will be of the mucopurulent variety, the pus being for the most part in streaks or balls. In the latter, the so-called nummular sputum, older authorities saw certain evidence of phthisis, or even of cavities. But bacilli may be found in sputum of any kind, so that we must not be guided too much by macroscopic characteristics. By spreading the sputum out with glass needles we make out the presence of any unusual elements, as blood, or the small particles to be described later. Blood, even in minute quantities, can generally be detected in this rough way. Even if we find nothing unusual in this search, it is well to examine some of the more opaque parts under the microscope without any reagent. In this way we satisfy ourselves as to the condition of the pus corpuscles, and of the presence and character of the so-called alveolar epithelial cells. These latter are large round or oval cells containing a clear nucleus, and black or brown pigment or fat particles. Of no special importance in the diagnosis of phthisis, they indicate the pulmonary element in sputum in doubtful cases. The macroscopical particles mentioned above are of great practical value. Large, irregular, whitish or pinkish masses are usually fragments of food or else filiform papillae from the tongue, with enormous quantities of bacteria. The larger mass here is made up of colonies of bacteria

which grow in a close, felted arrangement so that at first glance it has a fibrous appearance. This might easily be mistaken for elastic tissue, but pressure on the cover glass causes the strands to break up and to show the individual bacteria. Of much greater interest are small grayish or yellowish white particles which, when carefully studied can usually be recognized with almost certainty. Picking up one or two of these, placing them on a slide and examining under a low power we see a network of dark fibres and a quantity of cells and granular matter. The fibres are those of elastic tissue and they can be studied better by running some dilute caustic potash under the cover-glass. This dissolves out the cells so that the fibres can be seen alone. With the low power we can see the fibres are arranged in a mesh-work which we recognize as inter-alveolar fibrous tissue. The higher power shows the characteristic curling and branching, sharply contoured fibres. In this permanent preparation you can see elastic tissue in a less common form. You see here an oblong plate with narrower processes branching off at an acute angle, and only at the edges can you see single fibrils. This we recognize as a piece of the fibrous coat of a bronch'ole. The finding of pulmonary elastic tissue in sputum is of the greatest diagnostic value. Formerly it was looked for in all cases of suspected phthisis, but since the discovery of the bacillus has taken a less prominent position. To omit looking for it, however, is inexcusable, since its presence in sputum indicates conclusively that a destructive process is going on in the lung. Not necessarily a tuberculous process, since it may be found in abscess, in non-tubercular bronchiectatic cavities and sometimes in gangrene. Most frequently in the latter disease it is not found, being dis-

solved by a ferment formed in the gangrenous process. Combined with the finding of tubercle bacilli it is of course of great import. But it is only when we can trace the pulmonary structure that it can be used as evidence. Single fibres can come from food particles, elastic tissue remaining unaltered in the mouth during several days. Single fibres of elastic tissue are sometimes simulated by long, narrow needles of fatty acids. Heating the slide causes the latter to disappear.

As you have seen it is not difficult to find, and I think the method shown you the most practical. Fenwick's method, formerly used, consisted of boiling sputum with dilute caustic soda and examining the undissolved residuc. It is generally held that elastic fibres do not occur in sputum as early as bacilli. Indeed, I know of but one authority, and a very good one,—Troup,— who maintains the contrary. I have never found elastic tissue first, although I have looked for it in many hundred cases, never, I must admit, by boiling or for single fibres. But in many cases, as in the case of P. H. cited above, I have been able to examine the entire expectoration, even under a high power, without unusual expenditure of time, and with negative results.

Sometimes in the opaque masses containing elastic tissue we find giant cells. These do not show their branching processes, but have smooth outlines, as in the drawing.

We come now to the most important part of the sputum examination, that for tubercle bacilli. The possibility of recognizing these bacilli depends on their peculiar reaction to certain aniline dyes and reagents. As you know bacteria in general share with nuclei an affinity for certain dyes, especially those aniline dyes known as basic. So when we treat a preparation containing cer-

tain bacteria with a solution of such a dye, and wash away the excess with an indifferent fluid, the bacteria retain the color. I say an indifferent fluid, for if we used, say, a dilute acid, the bacteria too would be decolorized. The tubercle bacilli are peculiar, in that they stain with great difficulty, but when once impregnated with the dye they retain it with tenacity. In this respect they are almost unique. But one other bacterium, that of leprosy, possesses this peculiarity, but as that can be stained by simpler methods, and for other reasons can be excluded from such examinations as ours, we can speak of the stain as a specific one. Many methods or modifications of methods are in use; the best is the one devised by Ehrlich and adopted very early by Koch. In this, the coloring solution is made by dissolving the dye in a solution of aniline oil, a basic coal-tar product, and itself the source of many dyes,—and decolorizing by dilute nitric acid.

For staining tubercle bacilli in sputum the method is as follows: We pick out a small quantity of the denser part of the sputum, one of the small opaque particles, if possible, avoiding the pure mucus. This we place on a cover glass, press another on top so that the sputum will be spread out in a thin and uniform layer, draw the covers apart and let them dry, sputum side up, in the air. When dried they must be heated, in order to make the layer homogeneous and prevent its washing off in the subsequent operations. Experience has shown that the proper degree of heat may be secured by passing the covers over the flame of a Bunsen's or spirit lamp, "about as rapidly as one waves a handkerchief in greeting." In the mean time the staining solution has been prepared in the following manner: An excess of aniline oil (soluble in the proportion of about 1:30) is shaken

with distilled water and the turbid fluid passed through a moistened filter paper. To the clear filtrate we add a saturated alcoholic solution of gentian violet, methyl-violet or puchsia (known also as magenta), until an iridescent metallic pellicle forms on the surface. The covers are placed in this, either submerged, or floating with their coated sides down, and left at the ordinary temperature for a day, when they are stained, and ready for decolorizing. The process may be shortened by heating the solution, either in a watch-glass held above the flame or by running the stain on the cover-glass and holding it above the flame until vapor rises from the surface. The next step is decolorizing. We take a cover-glass by one corner, drain off rapidly most of the stain, immerse it in a ten per cent. solution of nitric acid until all the color is changed to a greenish yellow, then pass it through seventy per cent alcohol. This at first restores the color, and we wash to and fro until no more clouds of color come away and the glass is almost clear. A faint general tint remains if the bacilli are present in very large numbers. The specimen can now be examined in water, or may be dried and examined in oil of cedar or permanently mounted in balsam. It is often advisable to make a double stain, to stain nuclei or other bacteria in the preparation,—an assistance in searching for bacilli when few. We use a contrasting color; brown in the case of blue primary stains, and blue or green for the red. A watery solution (2 per cent) of Bismark Brown, methyl-green or aniline blue is allowed to act on the sputum for a minute or two and then washed off with distilled water. The cover can then be examined or dried and mounted as above. For the examination a good microscope is necessary. A good $\frac{1}{4}$ inch lens will show the bacilli

clearly, or even a $\frac{2}{3}$ inch in good preparations. But in case of uncertainty the best lenses, (oil immersion) are necessary, and the concentrated light furnished by an Abbe condenser. The bacilli appear as you see them, as slender rods, straight, curved or bent, varying in length from one-fourth to one-half the diameter of a red corpuscle, and lying singly or in groups. Sometimes clear spaces, supposed to be spores, can be seen interrupting the colored lines.

Simple as this process seems, and is when the technique is mastered, it is something which demands care and skill. There are many sources of error and no one should attempt to give an opinion on phthisical sputum who has not qualified himself by extensive practice in that part of microscopical diagnosis. It is best to begin by examining a sputum known to contain bacilli, or still better some of the matter from a tubercular cavity, and to stain many dozens or even hundreds of covers before assuming the responsibility of reporting on an unknown case.

The most important causes of failure are: poor selection of sputum; too thin or too thick a layer on the cover-glass; over, or under heating; improper preparation of staining solution (to be avoided by keeping "standard" covers on hand and testing when necessary); insufficient staining; excessive decolorization by acid or alcohol. Here "practice makes the master."

In case the bacilli are found we have certain evidence of the existence of a tubercular process somewhere, the further elaboration of which must be deferred to another time, as must also, since it is not a laboratory topic, a discussion of facts bearing on the prognosis of tuberculosis. If we find no bacilli after careful search our task is a delicate one. But the search must be close. If we are sure we have a pulmonary

sputum we can not go to too much trouble, and the same is true if for any reason we have to examine a poor specimen. I have examined twenty slides from a large amount of saliva sent a long distance, without result, but in the twenty-first found a number of bacilli. In case we find no bacilli we are warranted only in saying that, and not in general terms saying there is no tuberculosis. For we know that in some cases of general miliary tuberculosis there is no break-down and consequent expectoration of tuberculous material, at any period, and in some cases of rapid phthisis only toward the end. When in chronic cases bacilli remain persistently absent we are justified in believing the disease is non-tuberculous.

The method I have given you is the one you should begin with, and always use in cases of importance. Of the many modifications of it there is one largely used by clinicians and investigators on account of the rapidity and certainty of its action, and the permanence of the staining solution. This is called the carbol-fuchsin method, or from the name of its originator, Ziehl's. The solution is prepared as follows: To 90 parts of a saturated solution of carbolic acid add 10 parts of alcohol in which one part of fuchsin has been dissolved. The mixture is turbid at first, but becomes clear in a day or two and can then be used. The stability of the solution can be seen in this bottleful, which was made more than a year ago and is still effective. The other procedures are as given for the Koch-Ehrlich method. With this solution cover-glasses heated as I showed you can be stained within a minute. The solution also acts rapidly in ordinary temperatures and I have stained the bacilli in a tuberculous supra-renal body in twenty-five minutes with it. While the object of the demonstration was to show the method for sputum examinations, the general principles apply to all fluids suspected of containing tubercle bacilli, such as urine, pus, exudates, etc.

