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To facilitate the examination of sputum for tubercle bacilli, and to render more conclusive such examinations when but few bacilli exist in a given specimen of sputum, as well as to offer a substitute for the complicated methods now in vogue for the detection of fibers of elastic lung-tissue, are the objects of the present paper. When sputum contains tubercle bacilli in but scanty numbers it frequently happens that repeated examinations are necessary before their presence can be satisfactorily demonstrated; and even then their existence at times fails of detection, as will presently be shown, when by the aid of the centrifuge that they exist in such sputum can be readily made clear.

So also the application of centrifugal force, in the manner about to be described, to the detection of elastic fibers will be found a great improvement over

the present methods. For this purpose at present the plan most generally adopted is that advocated by Fenwick, which consists in boiling the sputum in a dilute solution of caustic potash, then allowing it to settle for twenty-four hours, and examining the sediment for elastic fibers under the microscope. To those who are in the habit of employing this method it is not necessary to call attention either to its complications or to the uncertainty of its results, both of which render its employment practically useless in the ordinary routine of clinical work. Nor is the simpler method advocated by von Jaksch, of adding some potash solution directly to the sputum as it is placed upon the slide, with the object of dissolving the pus and allowing the elastic fibers to be seen, to be any more strongly recommended, as it is open to the chief objection that characterizes Fenwick's method, viz: its uncertainty, and the number of examinations usually necessary before, if at all, the elastic fibers can be found.

The observations about to be described were made from sputa taken from patients presenting themselves at the out-patient medical department of the Jefferson Medical College Hospital; and because in the present paper it is desired to describe the effect of centrifugation only as it may apply to the tubercle bacillus and elastic fibers of lung tissue, none other than tuberculous sputum is included. This is done, too, notwithstanding that sputa belonging to other diseases of the lungs have been similarly treated, as, for example, the sputum from cases of bronchial asthma, in which we have found

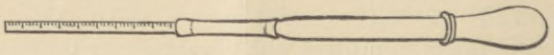
that Charcot-Leyden crystals and the spirals of Curschmann can also be very readily demonstrated.

The mode of procedure is as follows: The instrument utilized to produce the required centrifugal force is a machine made by F. F. Metzger, of Philadelphia, under the direction of Judson Daland, as a modification of the hematokrit of Blitz-Hedin, with the difference that the caliber of the glass tube in which the precipitation is to take place is two-and-one-half millimeters in diameter, instead of one-half millimeter, as is the case with the hematokrit. In both instruments the length of the tube is the same, about fifty millimeters. Inasmuch as the greater the number of revolutions of the precipitating tube we are able to produce in a given time so much less time will be required in the manipulations, thereby simplifying the procedure, the machine made by Metzger is advocated, as no instrument yet brought to the attention of the writers has been found to approach it in speed. Though as yet untried, the force derived from a small electric motor might be utilized with advantage for the purpose of furnishing the necessary power for the operation of the centrifuge.

The preparation of the sputum before placing it in the precipitating tube is extremely simple. A small quantity is placed in a porcelain dish, and actively stirred for a few moments with a glass rod. Under this treatment the sputum soon becomes, without the addition of any diluting fluid, a thin liquid, free from lumps, and apparently of homogeneous character. The precipitating tubes are

then filled, the sputum being drawn into them by means of rubber tubing having attached to one end a small rubber bulb to produce the necessary suction. We have used for this purpose an ordinary medicine-dropper with its attached bulb, inserting the nozzle end of the dropper into the end of the rubber tubing, the other end of which is applied over the end of the precipitating tube. This method has been adopted to render unnecessary suction by the mouth which, were there no other reasons why it should not be resorted to, might not be free from danger in the case of tuberculous sputum. For the purpose of elucidation a drawing of this simple apparatus is given, and so easy of application is this method that its use has been resorted to for the drawing up of the blood into the precipitating tube of the hematokrit, as it has been found that the column of blood is thus more susceptible of control than it is with mouth suction.

FIG. 1.



Medicine dropper attached by rubber tubing to precipitating-tube.

The two precipitating tubes filled with sputum thus prepared are now placed in the centrifuge, and rapidly revolved for at least two minutes. With a speed of ten thousand revolutions per minute, which is easily attained by the improved apparatus made by Metzger, two minutes is quite time enough to produce complete precipitation of the more solid por-

tions of the sputum, along with which, as shall presently be shown, go the objects of our search.

Precipitation thus having been accomplished, to the unaided eye the sputum will be found to have separated into two layers; that at the distal or peripheral end of the tube being yellowish or greenish in color; that at the proximal or central end appearing as a slightly turbid, almost transparent watery fluid, which by the microscope is found to contain no cellular elements whatsoever. In the peripheral end of the tube, however, the microscope reveals, closely packed together, but their integrity not in the least impaired, the various forms of epithelial cells and pus cells contained in the sputum. The more purulent is the character of the sputum, the greater the amount of desquamation of epithelium, of course the greater will be the depth in the precipitating tube of the purely cellular portion of the sputum—an observation possibly not without its value.

Undoubtedly the most important evidence that a destructive process is taking place in the pulmonary structure is to be found in the discovery of elastic fibers in the sputum. With obscure symptoms and signs pointing to a pulmonary tuberculosis, their significance is even greater than the presence of tubercle bacilli, unless in large numbers, as bacilli may be found in sputum when the lungs are in no wise diseased. The finding of elastic fibers is positively indicative of destruction, and the other destructive diseases of the lungs, such as bronchiectasis and pulmonary abscess, are not by their symptomatology likely to be confounded with tubercu-

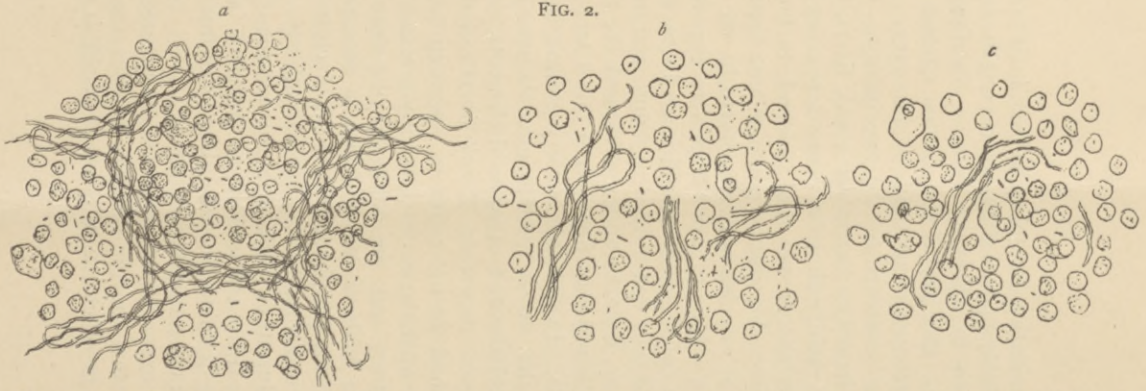
losis. Pulmonary gangrene is not here classed with diseases of the lungs characterized by destruction of the lung-tissue, and to be compared with tuberculosis, because, as was first pointed out by Traube, the sputum expectorated in cases of pulmonary gangrene rarely contains elastic fibers, for the reason that they are probably completely destroyed within the gangrenous area. Therefore, the presence of elastic fibers in the sputum, with signs and symptoms pointing to pulmonary tuberculosis, even though these be obscure, and even though the presence of the tubercle bacillus may not be demonstrated, would clearly establish the diagnosis of pulmonary tuberculosis. Hence, that which will supplant the complicated and uncertain method of Fenwick, or the somewhat less complicated but none the less uncertain method of Von Jaksch for the detection of elastic fibers, cannot fail to be of great practical value when the importance of the subject is taken into consideration.

From the large number of observations we have made, now amounting to many hundreds, it has been definitely demonstrated that elastic fibers are precipitated by centrifugal force in the largest numbers to the very extremity of the peripheral end of the precipitating tube. The sputum occupying this portion of the tube, when rich in cellular elements, is found to be quite thick in consistence, and is usually extruded as a mold of the interior of the tube. Without any dilution, or any means for the solution of the pus cells, a small portion of this sputum taken from the periphery of one of the tubes—the contents of the other tube being reserved

for the examination for tubercle bacilli—is spread somewhat thinly upon a slide, the cover-glass being pressed down to effect its equable distribution. Examined in this way, if the sputum contain any tissue its presence cannot fail of discovery. Examining the contents of the precipitating tube throughout its entire extent for elastic fibers, they are found to rapidly diminish in numbers as we pass from the periphery; and this is found to be the case even when the sputum contains lung-tissue in abundance, as was clearly demonstrated in one case especially, in which in the first slide were discovered ten separate collections or bundles of fibers, while in the second slide but three were found, and in the third but one, none being found beyond the third slide. The bundles of fibers were much larger in the first slide. In the thin, watery portion of the sputum, or that which remains after the precipitation of the cellular and other solid elements, elastic fibers are absent altogether. Elastic fibers examined by this method do not differ in appearance from those found after the methods usually practised for their detection, and the accompanying drawings of collective fields of sputum taken in succession, beginning at the peripheral end of the precipitating tube and proceeding toward the central end, present good illusrations both of the appearance of the cast-off lung-tissue and its relative frequency in the slides, as the examinations take place away from the periphery of the precipitating tube.

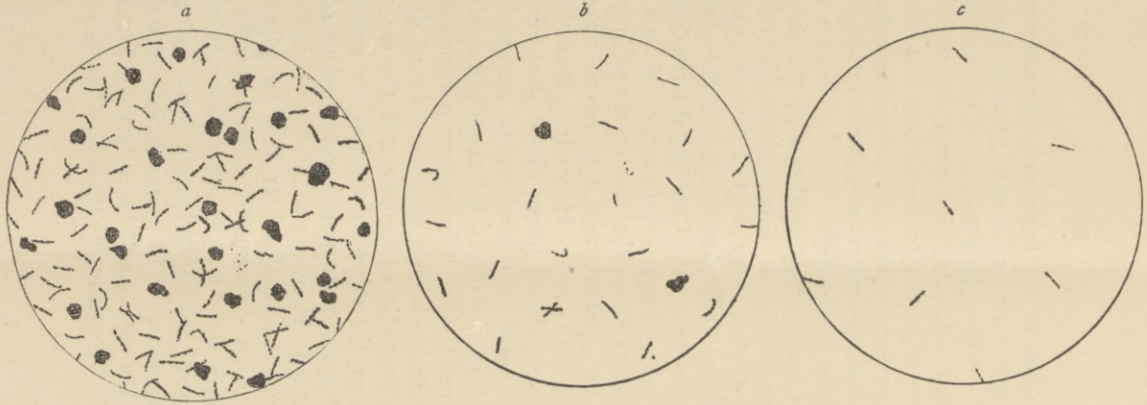
It is the experience of everyone accustomed to examine sputum for the tubercle bacillus that in those cases in which the number of bacilli is very

FIG. 2.



Elastic fibres. D objective, ZEISS. *a*. Slide prepared from extreme periphery of precipitating tube. *b*. Collective field from a slide taken 5 mm. from peripheral end of tube. *c*. Collective field from a slide taken 1 cm. from peripheral end of tube.

FIG. 3.



Tubercle bacilli. Homogen. immers. $\frac{1}{12}$, ZEISS. *a*. Slide prepared from extreme periphery of precipitating tube. *b*. Slide taken 5 mm. from peripheral end of tube. *c*. Slide taken 1 cm. from peripheral end of tube.

small the result of the examination is often disappointing, and a number of such examinations have to be made before the presence of the bacilli can be demonstrated. Indeed, cases are not infrequently met with in which bacilli are not found in the sputum even after repeated examinations, and yet such cases will have presented well-marked evidences of pulmonary tuberculosis.

By centrifugal force, together with the pus cells and the various forms of epithelium, the tubercle bacilli are carried along. Thus it is that they are found in greatest abundance at the peripheral end of the precipitating tube. Centrifugal force does not separate them and assign them a place of their own, but they rather share the fate of the cellular elements of the sputum, which therefore carry the bacilli along with them. Hence it is, that with the bacilli as with elastic fibers, as we pass from the peripheral toward the central end of the precipitating tube they are found to rapidly diminish in numbers, until when the watery portion of the sputum is reached their presence fails of demonstration. That this is the case and the extent to which it is so, the appended drawings of slides prepared and stained from different portions of the tube will clearly show.

The value of centrifugal force as an adjunct to the examination of sputum for tubercle bacilli when they exist in only few numbers can scarcely be overestimated. Again and again have our observations demonstrated to us the facility whereby in such cases the presence of bacilli can be discovered by the aid of centrifugation, and in cases, too, in

which at the same time they were found only after much trouble and repeated examinations by the ordinary methods. This was especially noticed in a case in the wards of the Jefferson Medical College Hospital, which presented well-marked signs of advanced pulmonary tuberculosis, among them being the physical signs of a large cavity below the left apex anteriorly. In August, 1894, this case died, and the autopsy fully confirmed the diagnosis of pulmonary tuberculosis. The sputum of this case had been examined repeatedly by the ordinary methods, not only by ourselves but also by others expert in making sputum examinations, and at no time were bacilli found; and yet in this very case, by the aid of centrifugation bacilli were found in the sputum at the first examination, the number being very few.

In the course of these observations we have tried various ways of previously diluting the sputum, and have also considered the advisability of endeavoring to secure the solution of the pus corpuscles, thinking that by these means the tubercle bacilli and the elastic fibers would be more closely collected together.

We also subjected sputum to a series of examinations according to the method advised by Biedert, which, as described by Von Jaksch, consists of the following procedures: "Ten to twenty cubic centimeters of the sputum are boiled in a watch-glass with a little dilute caustic soda; water is added, and the mixture again boiled until it is thinly fluid. It is then allowed to stand in a conical glass for two or three days, after which the sediment is added to a small

quantity of egg-albumin, and examined for bacilli in the usual manner." Instead of allowing two or three days for sedimentation, the mixture was at once subjected to centrifugation, but the result was far from satisfactory.

In this connection reference should also be made to the method recently described by Ilkewitsch (*Centralblatt für Bakteriologie und Parasitenkunde*, Feb. 5, 1894, Bd. xv, Nos. 5, 6, p. 162), the preliminary procedures of which consist in mixing with a half cubic centimeter of sputum in a porcelain dish twenty cubic centimeters of distilled water. To this are then added eight to twenty drops of a 30 per cent. solution of potassium hydroxid, and the mixture warmed until vaporization occurs, until which time it is constantly stirred. Under the influence of the heat and the potassium hydroxid, complete solution of the sputum is supposed to take place, at which time a small amount of casein is added to the mixture and dissolved. The fluid is then poured into a test-tube and acetic acid added, drop by drop, until the first sign of coagulation of the albumin is observed. The liquid is then placed in the cylinder of the centrifuge and rotated until separation of its elements takes place. When the separation has occurred a small ball suspended by a thread is lowered into the cylinder upon the collected sediment, and the superimposed fluid allowed to flow out. The sediment is then treated according to the special method which it is the object of the paper of Ilkewitsch to set forth.

In the course of our work upon the subject we found that dilution of the sputum as a preliminary

measure to centrifugation is not only unnecessary, but interferes materially with the success of the examination, as it lessens the actual amount of sputum precipitated and diminishes the number of bacilli and elastic fibers available for observation, as well as increases the difficulty of staining. It is further rendered unnecessary because the sputum, by the process of trituration as above described, is rendered sufficiently fluid for proper manipulation; indeed, such sputum, even though abundantly rich in cellular elements, after centrifugation is found to be composed of more than one-half water.

The same objections apply to the various methods resorted to for causing a solution of the sputum, and it seems to us that such methods as those advised by Biedert and Ilkewitsch needlessly complicate what should be only a very simple process. The object of both of these methods is especially directed to the collecting together of bacilli, from sputum containing them in but scanty numbers, within a limited space. For accomplishing this purpose that method which is the simplest in its details, and which requires but a very few moments for its completion, provided that its object is effected with the same precision as by the more complicated means, is the method that will prove most practicable in the routine of clinical work. Furthermore, other things being equal, why should methods that are both complicated and time-consuming be followed when one presents itself in which both these objections are lacking? That we are not overestimating the value of the simple method just described, we think we have abundantly proved.

In looking into the literature of the subject we find but few references to the adaptation of centrifugal force to sputum examination, and the few obtained refer only to the precipitation of tubercle bacilli, and nowhere do we find recorded any observations upon the effect of centrifugation upon elastic fibers. We therefore feel that the observations made in the present paper, so far as they refer to this part of the subject at least, are original.

In conclusion we would say that, as a result of the above investigations, in the centrifugal machine we possess a very important adjunct to the present methods in use for the examination of sputum. Nor in its adoption will clinical medicine be burdened with a new instrument, as the centrifuge has so many well-demonstrated uses that in any clinical laboratory its presence is indispensable. In the form of the hematokrit its value is now acknowledged, and as a means for procuring the sedimentation of urine it possesses sufficient importance to warrant its possession were that the sole claim for its adoption; and, finally, that it is of practical value in the examination of sputum has been conclusively proven in the clinical laboratory of the out-patient medical department of the Jefferson Hospital, where for the past six months the routine examinations of sputum have been conducted with its aid.