THE SIGNIFICANCE OF THE EPITHELIAL CELLS AND SAPROPHYTES IN SPUTUM.

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PLATES 32 AND 33.

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Although text-books on diagnosis state that the source of epithelial cells in the sputum may be ascertained by their microscopic appearance, the significance of this fact is not emphasized.

The mouth and pharynx are lined with polygonal flat epithelial cells, and the vocal cords are covered with them, while the nasal cavities, larynx, trachea, and bronchi are lined with ciliated cylindrical epithelial cells. The bronchioles and bronchi are lined with cuboidal cells, while the alveoli are lined with polygonal cells[1] which, unlike those of the other organs, have no nuclei. If inflammation occurs in any of these organs, the cells are expectorated with the mucus, and the sputum must therefore contain the epithelial cells of the affected organ. But in chronic inflammation of the larynx, as in tuberculosis, the ciliated epithelial cells lining this cavity are supplanted by a new growth of polygonal flat epithelial cells like those of the mouth. This is shown by the continual occurrence of polygonal flat cells in the sputum of laryngeal tuberculosis. The ciliated cells are distributed over a wide area, but they scarcely ever appear in the sputum. I have only once detected ciliated cells. In sputum from the lungs round or elliptical epithelial cells, which are nearly twice as large as pus cells, are often present. They are the decomposed products of the cuboidal cells found in the bronchioles. They have vesicular nuclei and contain either particles of carbon or hemosiderin in their protoplasm, and correspond to

[1] Some authors believe that these cells are enlarged cuboidal cells, and not epithelial cells.

Wagner's heart failure cells. I have never found flat pulmonary epithelial cells without nuclei in sputum.

The saprophytes play an important part in determining the source of sputum. In spite of the fact that various kinds of cocci, bacilli, spirochetes, and spirilla are found in the nasal and mouth cavities and in the larynx and pharynx, the sputum from the trachea and lungs is always free from these non-pathogenic microorganisms. The polygonal flat epithelial cells are found with various saprophytes, while the sputa that come from the lungs or trachea contain only the agent of the prevailing disease, such as *Bacillus tuberculosis* or *Diplococcus pneumoniæ*. In cases of mixed infection, one or two species of bacteria are found. Large numbers of various kinds of microorganisms in the pulmonary or tracheal sputum were never demonstrated.

I shall refer to the sputum from the region below the vocal cords as pulmonary sputum, that from above, as laryngeal sputum, and the exudates of the nasal cavity as nasal secretions.

Technique.

The same patient sometimes expectorates sputa of different appearance, revealing the various degrees of inflammation or other conditions of the affected parts. All the different sputa must therefore be examined, and the material for examination must be fresh and moist. A specimen attached to a piece of paper or gauze is unsuitable, as shown by the following experiment. A film preparation of a specimen of sputum to which some powdered starch had been added was pressed gently with a piece of blotting paper. The specimens were treated with iodine, stained with dilute fuchsin, and examined under the microscope. The particles of starch were found to be completely mixed with the sputum. On the other hand, the sputum that had been mixed with the starch suspension in salt solution and stirred vigorously proved on microscopical examination to have been mixed completely, and when subjected to the method of washing shown below, no particles of starch were demonstrated. This shows that the dehydrated starch grains stick to the sputum and cannot be washed away, while grains that have been mixed while

still moist with sputum are completely washed away. It is therefore clear that sputum from the lungs will be mixed with flat epithelial cells or some non-pathogenic microorganisms on its way to the mouth, but if the sputum is moist, the mixed elements will be completely washed away by the following method.

Washing.—A small specimen of the sputum is attached to the end of a platinum wire and immersed in a test-tube filled with isotonic salt solution. The material should be thoroughly rinsed with the platinum rod until it breaks up into several still smaller pieces. By washing the material once in this manner, it will usually be freed from contaminating bacteria, but sometimes the same technique must be repeated with fresh isotonic salt solution before it is cleared. The material is taken out of the salt solution, dried, and is then ready for examination.

Film Preparation. Fresh Material.—For the purpose of demonstrating the blood in the sputum fresh material is most suitable. The material should be washed as described above and placed on the slide. The cover-glass is then pressed lightly with the tip of the finger in order to make the film of the sputum suitable for microscopical examination.

Stained Material.—A small specimen of the washed sputum is put upon the middle of the slide and covered with a cover-glass, which is pressed lightly with the tip of the finger in order to obtain thin films. The cover-glass is then immediately pushed aside in contact with the surface of the slide, and removed, allowing the material to be dried in the air. If the material should be smeared with the platinum rod, the preparation is likely to be contaminated by the microorganisms attached to the rod.

Fixation.—For the demonstration of the blood in the sputum, the material is fixed by immersing it in absolute alcohol for 30 to 60 minutes, or in pure methyl alcohol for 15 minutes. For the examination for tubercle bacilli the material can be merely passed over the flame. Care should be taken not to let the material become too hot, as this destroys the staining properties of the pus cells and other elements.

Staining.—The blood is demonstrated by the same method that is employed in the preparation of the stained blood film. For the

examination of the bacilli, Ziehl's carbol-fuchsin is used, which is then decolorized with a mixture consisting of 100 cc. of absolute alcohol and two drops of highly concentrated hydrochloric acid. By this procedure, the erythrocytes lose the hemoglobin content and have the appearance of a blank vesicle. Löffler's methylene blue may be substituted by borax-methylene blue, which is prepared by mixing 1 gm. of methylene blue with 2 gm. of borax and 100 cc. of distilled water. The mixture is left standing for over 2 months and is then ready for use. It may be still further diluted to 10 per cent. It is superior to Löffler's preparation, for the cells as well as bacilli take deeper stains, while not only the original mixture, but also the dilution can be kept for a long time.

Diagnosis.—The most important data by which the source of the sputum can be determined are as follows: the erythrocytes or bacilli must be included in the same membrane of the mucus with other elements of the sputum such as the epithelial cells, contaminating microorganisms, or white corpuscles, which indicate the origin of the sputum itself. The diagnosis can be established only when these bodies are found closely packed in the same membrane of the mucus.

Pulmonary Sputum.—(Figs. 1 and 3.) Pulmonary sputum may sometimes contain round or elliptical epithelial cells a little larger than pus cells, but it never contains polygonal flat cells with nuclei. Theoretically, polygonal flat cells without nuclei may be found in the pulmonary sputum, but I have never observed any. Excepting the cases of mixed infection, one, or rarely two species of bacteria besides tubercle bacilli may be found. In no case does it contain an abundant number of different species of bacilli. If the clean sputum contains erythrocytes, the diagnosis of pulmonary hemorrhage may be made. If the same specimen should prove positive for tubercle bacilli by staining, the hemorrhage may be concluded to have occurred in the tuberculous lesions in the lungs.

Laryngeal Sputum.—(Figs. 2 and 4.) Laryngeal sputum usually contains polygonal flat cells with a large number of numerous species of microorganisms. It may contain only a few of these elements, but very rarely. If the laryngeal sputum contains erythrocytes the hemorrhage has occurred above the larynx. Again, if it is found to

contain tubercle bacilli, the diagnosis will be laryngeal or pharyngeal tuberculosis.

Nasal Secretions.—The nasal secretion of an acute nasal catarrh never contains flat epithelial cells or ciliated cylindrical epithelial cells, but may present the features of pulmonary sputum under the microscope. It usually contains, unlike the pulmonary sputum, various species of microorganisms, but sometimes contains only a few cocci or influenza bacilli. In such a case, it is difficult to establish a diagnosis by microscopical examination. In chronic nasal catarrh polygonal flat cells are always met with, but sometimes the material may contain only a few non-pathogenic contaminating bacilli. The secretion of the nasolaryngeal cavities always contains flat epithelial cells and various species of microorganisms, and presents the same features as the pharyngeal or laryngeal sputum.

By the method of examination described above, the source of the sputum can be determined and the diagnosis is thus facilitated. After several washings the material becomes free of the extraneous elements which may have come in contact with it; *e.g.*, polygonal epithelial cells. These polygonal flat epithelial cells under such circumstances will be found to be few in number, seldom exceeding two or three, and different in appearance from those of the laryngeal sputum. Moreover, in the pulmonary sputum these flat cells do not stick together with the tubercle bacilli or erythrocytes. By careful treatment and washing of the material, reliable results may be obtained. Saliva will be dissolved by the isotonic salt solution when it is washed, and completely separated from the materials together with any solid elements that might have been contained in it. If, again, two specimens of sputa are placed in contact with each other, they will not stick together unless they become dry. By washing a specimen of moist sputum, all the attached particles will be completely separated and removed from the material. By this means error in examination is avoided. If all the erythrocytes are removed by washing the material several times, the source of the hemorrhage is above the larynx.

I detected erythrocytes in the pulmonary sputum of all cases of hemoptysis examined immediately after pulmonary hemorrhage. The sputum from patients with laryngeal tuberculosis showed tubercle

bacilli in every instance. Laryngeal sputum is often so difficult to obtain that a number of examinations are usually required. Large masses of laryngeal tuberculosis sputum are seldom found; the specimens are usually small and mixed with saliva. A diagnosis, therefore, cannot always be established even after two or three examinations. From patients with both laryngeal and pulmonary tuberculosis two kinds of sputum are obtained and both must be examined. I have diagnosed the early stage of laryngeal tuberculosis in 6 out of 150 patients in whom no symptoms of the disease were evident.

Bacteriological examination of the laryngeal sputum of frank cases of laryngeal tuberculosis frequently showed tubercle bacilli; in some cases, however, scores of examinations were made before the contaminated sputum was obtained.

SUMMARY.

1. Microscopical examination of sputum that has been washed with isotonic salt solution indicates its source. Sputum from above the vocal cords contains polygonal flat epithelial cells and numerous species of non-pathogenic microorganisms. Sputum from below the cords is clear of saprophytes, although it sometimes contains bronchoalveolar cuboidal cells.

2. The source of the sputum can be determined by the erythrocytes and the bacilli that are contained in it, and the site of the lesion can also be ascertained.

3. In every instance I found erythrocytes in the pulmonary sputum after severe pulmonary hemorrhage.

4. Six of the cases of laryngeal affection referred to in the present paper had had no subjective symptoms, but microscopical examination showed tubercle bacilli in the laryngeal sputum. All the six cases were examined by Dr. Tanaka and were found to have tuberculous lesions in the larynx. In the laryngeal sputum of most cases of laryngeal tuberculosis with symptoms, tubercle bacilli have been frequently demonstrated, while in some cases contaminated laryngeal sputum was obtained only after a long series of examinations.

5. Microscopical examination of the sputum plays a significant part in the diagnosis of tuberculosis.

In conclusion, I wish to express my indebtedness to Dr. Tanaka, laryngologist, who supervised the laryngeal examinations for me, and to Messrs. Nemoto and Shiiba for their assistance.

EXPLANATION OF PLATES.

PLATE 32.

FIG. 1. Sputum from a patient who had a pulmonary hemorrhage (about 30 cc.); fresh specimen. Leitz oc. 1, obj. $\frac{1}{12}$, oil immersion. The stained preparation from the same material shows tubercle bacilli corresponding to No. V of Gaffky's scale.

FIG. 2. Laryngeal sputum from a patient with laryngitis; fresh specimen. Leitz oc. 1, obj. 4. Numerous polygonal flat epithelial cells and a few white corpuscles are seen. The stained preparation of the same material shows also numerous saprophytes. No tubercle bacilli were seen.

PLATE 33.

FIG. 3. Pulmonary sputum. Stained preparation. Leitz oc. 1, obj. $\frac{1}{12}$, oil immersion. White corpuscles, tubercle bacilli, and a cuboidal cell are seen.

FIG. 4. Sputum from a patient with laryngeal tuberculosis. Stained preparation. Leitz oc. 1, obj. $\frac{1}{12}$, oil immersion. White corpuscles, most of which have been destroyed, and tubercle bacilli are seen with the polygonal flat epithelial cells and numerous saprophytes, especially cocci and bacilli.

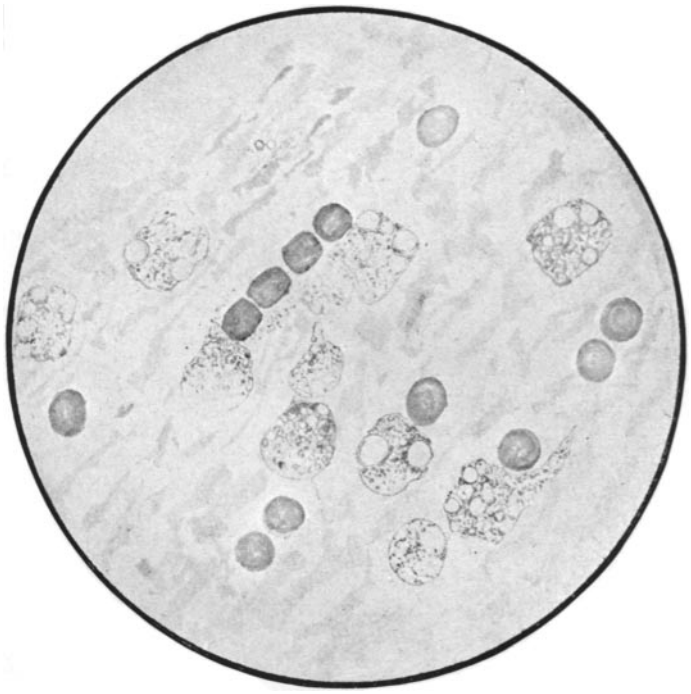

FIG. 1.

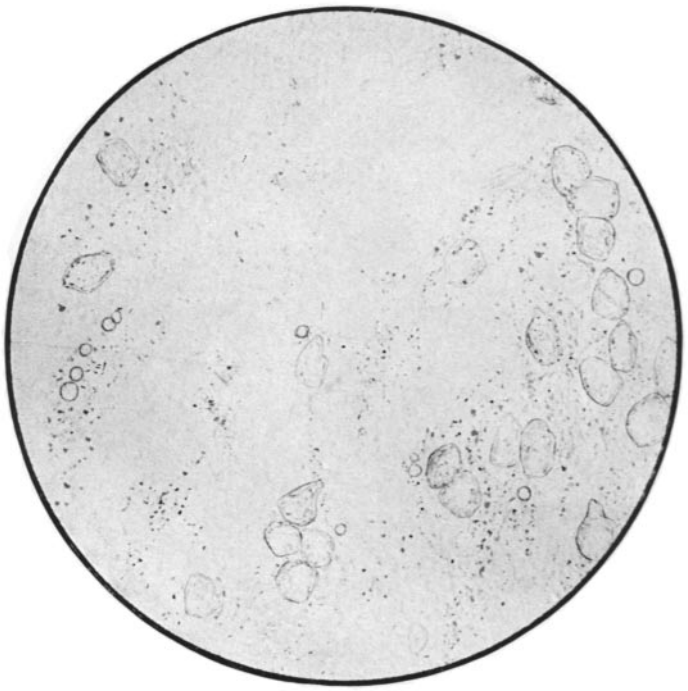

FIG. 2.

(Otani: Epithelial Cells and Saprophytes in Sputum.)

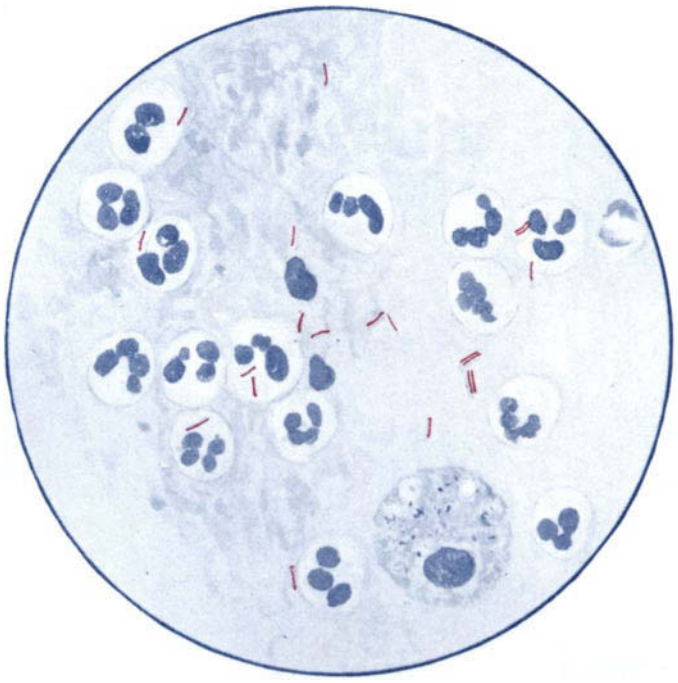

FIG. 3.

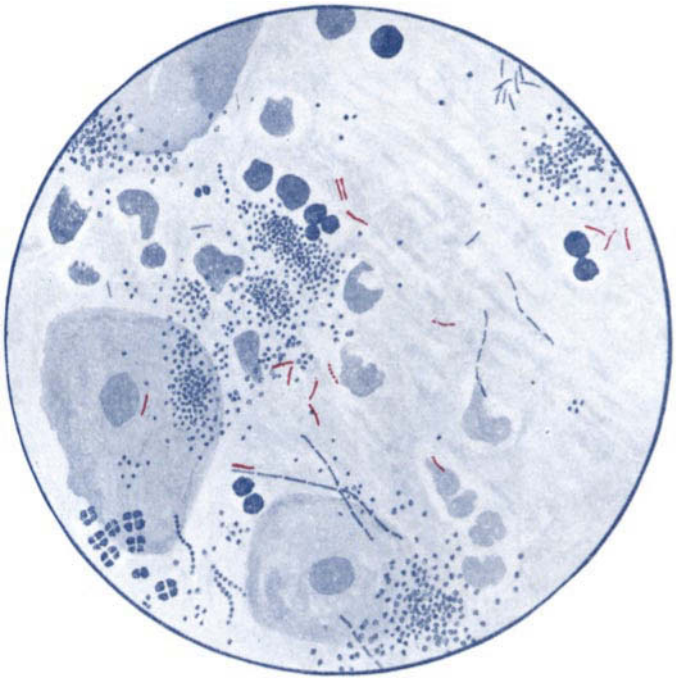

FIG. 4.

(Otani: Epithelial Cells and Saprophytes in Sputum.)