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THE CYTOLOGY OF SPUTUM

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Although the Oxford dictionary describes sputum *inter alia*, as saliva, it is usually understood by the medical profession to mean bronchial secretions only. This is the meaning in which the word will be used here. The word saliva, on the other hand, will be used to refer to the secretion in the mouth which is largely the product of the salivary glands.

DUDGEON'S METHOD

There are many ways of demonstrating the cells of sputum. After trying a number of stains and techniques, however, we have to fall back on Dudgeon's method, which, since it does not appear to be generally known, will be described here at length. Several smears from the sputum are made on clean glass slides. These, while still wet, are placed in Schaudinn's solution, the formula for which is: Saturated mercuric chloride in ·85% NaCl 2 parts, 95% ethyl alcohol 1 part, acetic acid a few drops. The chloride solution is stable but must be added to the alcohol and acetic acid each day.

The smears are allowed to remain in this solution for 20 minutes in order to secure complete fixation. They are then transferred to a jar of methyl alcohol to which a few drops of tincture of iodine have been added to remove the mercury. The smears remain here for 5 minutes. Then, after a rinse in water, they are placed in Meyer's haemalum for a period of 3 to 4 minutes. The time will vary depending on the condition of the haemalum. When removed they are rinsed again in water and 'blued' in a 1% solution of ammonia. This is followed by another rinse in water after which they are placed in a solution of erythrosin for 1 to 2 minutes. Again, the time will vary with the condition of the stain. Then follows a bath in methyl alcohol for 2 minutes and then 2 successive baths in absolute alcohol each for 2 minutes, and finally they are placed into a bath of xylol. They may remain in the xylol for several hours, over-night if convenient, but not less than 3 minutes. They are then mounted in Depex. This procedure differs somewhat from the original method described by Dudgeon—mainly in that erythrosin is substituted for eosin, ammonia for London tap water, and Depex for Canada balsam. Staining by this technique gives very fine nuclear definition which is all important in diagnostic cytology. Attention to detail is essential for first class results, and it is particularly important to remember that at no time during the whole process should the smears be allowed to dry. The entire procedure, from beginning to end, can be completed in less than an hour.

Apart from blood cells, which will not be considered here, cells of several different kinds appear in sputum. Some of these defy identification but the majority are usually easily identified. It would seem reasonable, in a paper such as this, to classify the cells in the first instance into normal and abnormal categories. Though apparently logical, such a classification would not exclude criticism for the reason that because sputum is itself an abnormality, its cell content must be abnormal. Because of this, and also because the main function of a cytological examination of the sputum is the detection of malignant cells, it seems better to classify the cells into benign and malignant groups.

TYPES OF CELLS

The following are the cells most frequently found in sputum in cases other than those of bronchial cancer. They are of course also found in bronchial cancer. In each case the description is based on the appearance of the cells in preparations of sputum treated by the above technique, and all drawings and photographs are taken from such preparations.

Simple superficial squamous cells (Fig. 1). Although almost invariably present in specimens marked sputum, these cells usually have their origin in the saliva. It is easy to identify them. They are amongst the largest cells found in sputum; they have abundant cytoplasm which stains faintly with eosin; their nuclei are round or oval and are centrally placed. These nuclei have a fine chromatin arrangement and usually stain fairly faintly with haemalum. On occasion they are pyknotic. In their general aspect these cells present the appearance of a flimsy, delicate structure, they are usually polygonal, but sometimes rounded or roughly triangular in shape. They seldom if ever give rise to confusion.

Superficial bronchial epithelial cells (Fig. 2). In appearance this variety is elongated and rather rectangular or triangular with a narrow base. Cilia may or may not be seen arising from one pole. The cytoplasm stains irregularly with eosin and often shows vacuolation. Sometimes this vacuolation is so great that the cell assumes a rounded rather than a rectangular

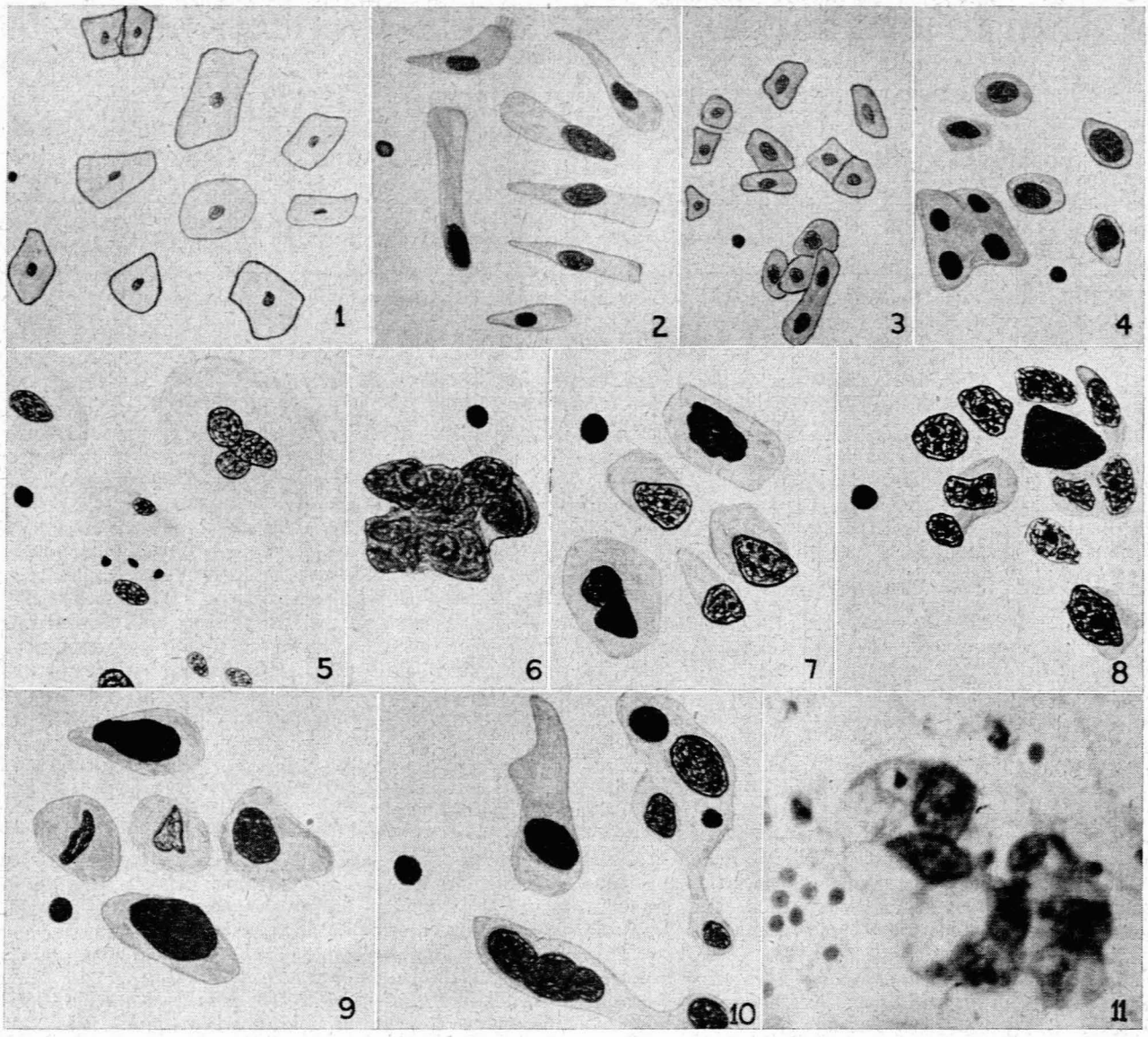

Fig. 1. A group of simple superficial squamous cells. (The separate black dot in this and the other drawings is a red cell drawn to scale.)

Fig. 2. Superficial bronchial epithelial cells. Cilia are apparent on one of them.

Fig. 3. Intermediate bronchial epithelial cells. Note that the cytoplasm is not as abundant as in the cells in Fig. 1, but more abundant than those in Fig. 4.

Fig. 4. Deep epithelial cells. These cells are easily confused with malignant cells; the two on the right might pass as examples of malignant cells.

Fig. 5. Histiocytes. These may take on various forms. Those depicted here are fairly representative.

Fig. 6. A group of malignant cells. Note how the nuclei overlap each other in a disorderly fashion, and also the paucity of cytoplasm. This case was proved histologically.

Fig. 7. Individual malignant cells. The nuclear irregularity, the prominence of nucleoli, and the increase of the nuclear chromatin, are the outstanding features of these cells. This case was proved histologically.

Fig. 8. Individual malignant cells showing features similar to those in Fig. 7. Note the absence of cytoplasm in some of the cells. This case was proved histologically.

Fig. 9. Individual malignant cells from a case diagnosed in the first instance by cytological methods, and subsequently confirmed by biopsy. Hyperchromatism of the nuclei is prominent but the cell in the centre is hypochromic.

Fig. 10. Further variations in the appearance of malignant cells. Note the tendency for nuclei to share a common cytoplasm.

Fig. 11. A photomicrograph from the same case as Fig. 10. Because of the third dimension it is not possible to have the entire picture in focus. The malignant characters can nevertheless be seen in this group.

appearance. The nucleus is situated near the pole remote from the cilia, it is round or oval and usually has a vesicular character. Palisading of these cells is common, but even apart from this they are often found in groups.

Intermediate epithelial cells (*Fig.* 3). These cells are more compact than the superficial variety, but they nevertheless bear a strong family resemblance. Their cytoplasm is abundant but not as abundant as in the superficial cells, and the cytoplasm stains more deeply with erythrosin. In comparison with the superficial cells the nuclei are larger in relation to the size of the cell, and usually they stain more vividly. These cells may be confused with histiocytes or with malignant cells. They are distinguished from the former by the fact that they have more erythrosinophilic cytoplasm, and from the latter by the character of the chromatin in their nuclei.

Deep epithelial cells (*Fig.* 4). These bear the same relationship to the intermediate cells as the intermediate cells bear to the superficial cells. They are squat and contain a large nucleus in comparison with the size of the cell. The cytoplasm stains vividly with erythrosin and the nucleus deeply with haemalum. They may thus appear very like malignant cells with which they are very easily confused. The nucleus, however, is usually normal in size, shape, chromatin content, and chromatin distribution. These characteristics of the nucleus distinguish them in most cases from malignant cells. Nevertheless, they constitute a real hazard in diagnostic cytology. Papanicolaou,[1] in his excellent atlas, illustrates and describes cells which are known in his laboratory as 'PaP' cells. They were discovered in his own sputum while he was suffering from chronic inflammation of the upper respiratory tract. One group of them (number 28 C 1 respiratory system) has the appearance of deep epithelial cells. They can thus be easily mistaken for malignant cells.

Histiocytes (*Fig.* 5). With the exception of the simple superficial squamous cell, these are generally the largest cells found in sputum. The cytoplasm stains pinkish with erythrosin, it is nearly always abundant and often appears foamy. Sometimes it stains so faintly that it can hardly be seen. Inclusions are very commonly found in the cytoplasm; these may be particles of carbon, red cells, or even malignant cells. Sometimes the inclusions are so bulky that they appear almost to burst the cell. The nucleus is either round or oval, centrally or eccentrally placed, and sometimes multiple. The nucleus usually presents a fine network of chromatin in which the nucleoli are often prominent. Sometimes the chromatin arrangement is dense and irregular and, since these are characteristics of malignant cells, there may easily be confusion. Fortunately some other feature of the cell, the abundant cytoplasm, or the inclusions, will distinguish it. Sometimes, however, these distinguishing features will not be present. A further confusing factor is that malignant cells themselves sometimes take on phagocytic activity, hence even the presence of phagocytosed material is not absolute proof that the cell is a histiocyte. Despite the fact that they constitute probably the greatest single hazard in diagnostic cytology, histiocytes are a welcome sight in the sputum because they indicate that the specimen probably came from deep down in the lungs.

Miscellaneous benign cells. Apart from blood cells, a number of other benign cells may be found in sputum. Some of these are vegetable in origin and sometimes bear a striking resemblance to malignant cells. Two of these are illustrated in Papanicolaou's atlas.[1] (C 11 respiratory system, number 12.) A study of their nuclei is the most important means of distinguishing them.

Malignant cells (Figs. 6-11). If there is one word which describes malignant cells, that word is variation. These cells vary in size, shape and staining characteristics. They may have single or multiple nuclei. The nucleus varies in size, shape, chromatin content and chromatin distribution. Nucleoli may be prominent or invisible; they vary in size, shape and position. Malignant cells may occur singly or in tightly packed clusters. It is this variation or irregularity in their general character that is the great distinguishing feature.

Individual malignant cells often, but not always, present the following features: The cell itself is big and the nucleus is big in comparison with the size of the cell. It is irregular in outline and its chromatin is irregularly disposed. Nucleoli are prominent and, as Dudgeon pointed out so many years ago, 'they often stand out as pink dots in the sphere of the nucleus'.[2] Hyperchromatism is an important criterion and this attribute often enables the cells to be seen under low power. Hypochromatism, however, also occurs, as would be expected, if malignant cells result from asymmetrical mitosis. Surprisingly, mitotic figures, normal or abnormal, are seldom seen.

There is thus no single morphological or staining characteristic by which malignant cells can be recognized with certainty, and, as was pointed out previously[3] no verbal description or illustration is adequate. Those who would pursue this subject, therefore, must study stained preparations for in that way only can the subtle details of the cells be fully appreciated. In this matter great help will be afforded by Papanicolaou's atlas.

Whilst in this paper, the cells of sputum have been classified in the first instance into benign and malignant groups, it must be remembered that in many cases this initial classification is by no means easy. There are many pitfalls, some of which have been pointed out by Koss and Richardson in their excellent paper on the subject.[4]

Furthermore, Bamforth, whose knowledge and experience in this matter is profound, stated in a personal communication that he would seldom, if ever, diagnose cancer on the appearance of a single isolated cell.

The view which most pathologists held until recently and which some still hold, namely that malignant cells cannot be distinguished from benign cells, is only partly true. In many cases this distinction can be made with confidence, though, it is also agreed, that in many cases it cannot.

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