THE CYTOLOGY OF PRIMARY BRONCHUS AND LUNG CARCINOMA

M. J. DE VRIES

THE CYTOLOGY OF PRIMARY BRONCHUS AND LUNG CARCINOMA

THE CYTOLOGY OF PRIMARY BRONCHUS AND LUNG CARCINOMA

PROEFSCHRIFT

TER VERKRIJGING VAN DE GRAAD VAN DOCTOR IN DE GENEESKUNDE AAN DE RIJKSUNIVERSITEIT TE LEIDEN, OP GEZAG VAN DE RECTOR MAGNIFICUS Mr J. M. VAN BEMMELEN, HOOGLERAAR IN DE FACULTEIT DER RECHTSGELEERDHEID, TEGEN DE BEDENKINGEN VAN DE FACULTEIT DER GENEESKUNDE TE VERDEDIGEN OP WOENSDAG 7 JULI 1954 TE 16 UUR

DOOR

MARCO JACOB DE VRIES

GEBOREN TE BATAVIA
(THANS DJAKARTA) IN
1927

1954

TER DRUKKERIJ VAN J. CAHEN N.V. 'S-HERTOGENBOSCH

Promotor:

Prof. Dr J. MULDER

Aan de nagedachtenis van mijn Vader
en mijn Grootvader, Arts J. Cahen

The investigation underlying this thesis was performed in the hematology laboratory of the Clinic for Internal Medicine (Professor Dr. J. MULDER, director), University Hospital, Leyden, Holland.

The histo-pathologic reports of biopsies, surgical specimens, and autopsies quoted in this work are from the Institute of Pathology (Professor Dr. G. O. E. LIGNAC, director), of the same hospital.

ACKNOWLEDGEMENTS

My sincere gratitude goes first of all to Professor J. Mulder, at whose suggestion I undertook this work. His stimulating interest greatly contributed to the completion of this paper.

To Dr. J. F. Ph. Hers, who collected and reviewed the pathologic reports, I express my best thanks for his continued help and encouragement throughout this study.

For his valuable advice I am indebted to Dr. P. Lopes Cardozo, who aroused my interest in clinical cytology.

I am very much obliged to Mrs. T. Bergeren van der Linden, whose cytologic skill was of great value to me. She and Miss H. Landman provided me with much technical assistance.

To Mr. C. C. A. Melchior I am especially grateful for the painstaking work of enlarging the photomicrographs.

To Mr. and Mrs. F. Gottlieb I am greatly indebted for the way they carried out the difficult assignment of translating the manuscript from the original Dutch.

I also wish to express my appreciation to Mr. M. Cahen for kindly undertaking the printing of this thesis.

In the course of this endeavour, my wife has been of constant, manifold assistance to me.

Finally, I offer my deepest gratitude to my mother, whose confidence and support enabled me to complete this work.

CONTENTS

Page

INTRODUCTION 11

CHAPTER I
Source of the material 13

CHAPTER II
Preparation of the material 18

CHAPTER III
Morphologic criteria of malignancy 29

CHAPTER IV
Normal cell morphology and some nonmalignant cytologic pictures of the respiratory tract 36

CHAPTER V
A cytologic classification of primary bronchus and lung carcinoma 44

CHAPTER VI
An evaluation of cytologic diagnosis 53

SUMMARY 59

REFERENCES 62

PHOTOMICROGRAPHS . . . . . .

INTRODUCTION

The use of cytology in the diagnosis of bronchogenic carcinoma found its way into various European and American clinics in 1940. The method, however, is a much older one, dating back at least as far as 1887. At that time Hampeln reported a case of pleural effusion, which postmortem examination later revealed to have been caused by a peripheral lung tumor. Five months previous to the patient's death, he had found cells in the sputum which he believed were derived from the tumor:

"The examination, which was performed about five months before the death, yielded, aside from debris and small, finely granulated cells, numerous polymorphic, club-, spindle-shaped, etc. cells of remarkable size, with granular content, and containing one or more nuclei . . . It is unknown to me that similar structures occur in sputum under normal or pathologic conditions of a different nature; at least I have never encountered them.

The diagnosis of a carcinoma communicating with the respiratory tract, based on the presence of such cells, seems justified and well founded." In a clinical lecture in 1897 Hampeln again stressed the importance of sputum analysis in the diagnosis of lung cancer.

Josselin de Jong (1928) reported in 1918, that he had found clusters of tumor cells in the sputum of two patients with malignant lung tumors. Again in 1929 he discussed three cases of pulmonary neoplasms where sputum analysis confirmed his diagnosis.

Vaandrager (1932) also recommended this method as a valuable aid in detecting pulmonary malignancy. In all of those cases where sputum morphology pointed toward lung cancer, the autopsy findings substantiated the diagnosis.

In their publication (1935) Dudgeon and Wrigley give an account of 58 cases of bronchogenic carcinoma. In 26 of these, tumor cells were evident in the sputum. The article also contains, for the first time, an accurately described cytologic technique.

Cytology as a means of detecting malignancy now came into fashion, and various workers, among them Wandall (1944), Herbut and Clerf (1946 and 1950), McDonald and Woolner (1947 and 1950), Farber et al. (1948 and 1950), published their findings. In 1949 there also appeared the paper of Papanicolaou and Cromwell on sputum

cytology. That same year The Journal of Clinical Pathology devoted an entire issue to this subject, reporting among others the contributions of Richardson et al. and those of Appel and Bronk on bronchus cytology. In Holland this method was studied by Swieringa (1949 and 1951), Lopes Cardozo and Janssen (1950), Keuning (1950 and 1951), Dijkstra (1951), and Deelman (1952).

In their paper Appel and Bronk (1949) describe their experiments with carcinomatous tissue transplanted into the bronchi of rabbits. Only six days after inoculation, at a time when bronchoscopic and roentgenologic examination showed no evidence of a malignancy, the bronchial secretions were positive for tumor cells in 17 animals (58 per cent). By the end of three weeks all animals were cytologically positive.

Clinical literature has vindicated the idea that bronchus and lung cancers of even the smallest dimensions can be discovered by cytologic means, provided they communicate with the lumen of a bronchus. The value of such an early diagnosis for the prognosis of the disease, combined with early surgical intervention, remained for a while undisputed. This view has recently been criticized by Korteweg (1953), who illustrated an old and well established clinical experience by means of statistics reported in recent foreign literature: the duration of symptoms, as measured from their onset up to the beginning of therapy, bears no appreciable relationship to the prognosis of the case. As a matter of fact, malignant tumors may have widespread metastases at a time when the primary growth is still so small that it causes no complaints from the patient.

A large number of bronchogenic carcinomas, however, belong to a pathologically more favorable group in which timely diagnosis affords a good chance for successful treatment. Cytologic analysis of sputum offers here the great advantage of being simple and not necessitating any unpleasant procedures on the patient. This is of special importance in the inoperable cases, where the patient may be spared needless alarm. At the same time, the technique is easy and may be constantly repeated. By the method of sputum analysis even those tumors which are inaccessible with the bronchoscope or biopsy forceps, can be subjected to a morphologic study.

Finally it must be remembered that should a causal therapy for cancer ever be discovered, the question of early detection will become even more urgent than it is today.

In this study we will report our experience obtained from cytologic examinations in 96 cases of bronchus and lung carcinomas.

CHAPTER I

SOURCE OF THE MATERIAL

Material to be examined cytologically can be obtained in three different ways; namely, from sputum, from the endobronchial channels, and by means of a directed lung puncture.

Sputum, because of rapid autolysis, must be used within the first three or four hours after collection. It is poured into one or more Petri dishes so that the floor is almost but not entirely covered, and then placed against a dark background. With the aid of two bistouries, the more solid parts of the sputum are removed. These usually consist of small pieces of tissue, necrotic or otherwise, hemorrhagic particles, or, in the absence of the latter, greenish mucopurulent parts. The samples are now distributed over several slides and smeared across the length of the glass. This is achieved by sliding the entire flat surface of another glass once over the material, the pressure used depending on the consistency of the specimen.

If wet fixation is employed (see next chapter), the slides, each of which has a paper clip at one end, are placed into a glass jar containing equal parts of 95 per cent alcohol and ether. With dry fixation, the preparations are left to stand for a while, but must be protected against flies.

The *endobronchial channels* offer us several opportunities for obtaining material for cytologic study. With the use of the *bronchoscope*, a biopsy or a curettage can be performed to obtain specimens from the main, lobar, and segmental bronchi. The tissue so obtained is brought in contact with a slide a few times, after which the "contact smear" is prepared as for sputum. The piece of tissue can then be fixed in formalin for further histologic examination.

If the tumor is so situated that it can be reached neither with a forceps nor with a curet, two alternate methods are at our disposal for obtaining material via the bronchoscope. A cotton swab can be

introduced through the focused opening of the suspected bronchus and applied to the mucous membrane, after which it is smeared onto a slide. The specimen will usually dry too quickly for a wet fixation smear, but we did use this method with dry fixation on numerous occasions. Bronchial secretions may be got by aspiration, as described by Clerf and Herbut (1950). If desired the bronchi can first be washed with saline. In either case the fluid is caught into a glass suction trap. After centrifugation at moderate speeds, the sediment is spread on a slide.

Neoplasms located in the peripheral parts of the lung or in the upper lobe at some distance from the origin of the superior lobe bronchus, cannot be visualized with the bronchoscope, and the help of a *Métras tube* may then be employed. Nolting (1951) and Swieringa (1951) have described this method whereby the tube is guided toward the site of the tumor with the aid of a fluoroscope. Once the tumor has been reached, a small forceps, a curet, or cotton swab attached to the end of a spiral wire, may be introduced through the tube. Smears are then made of the gained material. Aspiration of bronchial secretions may also be attempted by attaching a 20 cc. syringe to the end of the tube.

Where the above methods have repeatedly yielded negative results, and the conclusion has therefore been drawn that the neoplasm is a peripheral one and does not communicate with the bronchial tree, one more possibility remains open: *transpleural lung puncture.* Swieringa (1949) has described the historical background and the technique of this rather old method. The needle, which must be a thin one, is directed with the aid of roentgenologic visualization. It remains to be stated that a tumor which does not communicate with the bronchial tree but still seems operable is the only indication for this procedure.

As to whether the type of material has any influence on the number of positive diagnoses, many different views, some of them contradictory, have been expressed. Herbut and Clerf (1946) reported positive tumor cytology in 82.4 per cent of cases with bronchial secretions, whereas with sputum this percentage was always lower. Farber and Benioff (1948), on the other hand, found a positive sputum in 78 per cent of 23 cases of lung cancer in which both sputum and bronchial secretions were analyzed. Only 70 per cent had a positive cytology in the bronchial secretions.

Our own experience, derived from the examination of sputum and endobronchial material from 46 patients suspected of carcinoma, is presented in Table I.

TABLE I

The cytologic findings in 46 clinically suspected cases of bronchus or lung carcinoma, where both sputum and endobronchial material were available for examination.

	Sputum	Endobronchial material		
		+	±	−
+	21	21	−	−
±	9	5	3	1
−	16	11	−	5

+ = conclusive evidence of malignancy

± = suspected of malignancy

− = no indications of malignancy

Summarizing the facts contained in this table, we see that out of 41 patients found positive or suspected of malignancy via one or both methods, 30 (73 per cent) were diagnosed by means of sputum examination and 40 (98 per cent) by analysis of endobronchial material. Of 16 cases, where sputum cytology was negative, cancer was established in 11 by means of endobronchial material. One patient was negative in the latter examination but had a positive sputum cytology.

As far as material obtained by lung puncture is concerned, we have listed our findings on 16 patients with lung carcinoma in Table II.

TABLE II

The cytologic findings in 16 clinically suspected cases of bronchus and lung carcinoma, where both lung punctate and sputum were examined.

Lung punctate		Sputum		
		+	±	−
+	14	3	−	10
±	1	−	−	1
−	1	−	1	−

+ = conclusive evidence of malignancy

± = suspected of malignancy

− = no indications of malignancy

All the patients had tumors situated peripheral in the lung which could in no way be reached with the aid of the bronchoscope. In 11 the sputum was persistently negative, whereas in 3 it later became positive. In one instance a dubiously positive sputum could not be confirmed by lung puncture.

As a rule several different sources of cytologic material are at our disposal, each of which has its advantages and disadvantages. In a number of cases, however, one is restricted to the use of a certain material by the location and nature of the process.

If we consider the advantages of sputum examination relative to the other methods, the most important one will, no doubt, be the absence of any discomfort to the patient, so that it may be repeated as often as desired. Furthermore, sputum is a reservoir from which we can gain a general picture of conditions in the entire tracheobronchial tree without any instrumentation on the patient.

An important disadvantage of sputum analysis is the strong dilution of cellular material, which often makes the search for tumor cells time-consuming (an average of 45 minutes per specimen). Besides, many cells degenerate on their way through the bronchi to the outside, thus diminishing the reliability of the interpretation. In addition we have to contend with the difficulty that some patients with early bronchus or lung carcinoma have a nonproductive cough, so that this method of cytologic examination as an early diagnostic aid is not at our disposal. Of 84 patients who were bronchoscoped and of whom endobronchial material was examined, 38 coughed up no sputum or only pharyngeal sputum.

CHAPTER II

PREPARATION OF THE MATERIAL

Clinical cytology, in its broadest sense, has developed along two different lines and these are still reflected in the present-day methods of preparation.

Exfoliative cytology, a study which has been greatly expanded through the work of Papanicolaou, entails the use of techniques more or less derived from histology. Its field of investigation is limited to cells which loosen spontaneously and are transported via secretions or excretions through tubular organs to the outside. This makes it purely a tumor cytology, since it is chiefly the cells of a malignant tumor which display the property of exfoliating.

Puncture cytology, on the other hand, has a much wider scope, both pathologically and anatomically speaking. It was first employed in connection with clinical hematology and had, from the beginning, a more exclusive technique of its own.

In Holland it was especially Lopes Cardozo who tried to effect a synthesis of these two branches of clinical cytology by applying the same method of examination to both, thereby facilitating a comparison of the results.

A number of cytologists in Europe and South America, among them Richardson et al. (1949) and Deelman (1952), who devote their attention to exfoliating cells, restrict themselves to a purely histologic method. Sputum or bronchial secretions are fixed, enclosed in paraffin, and cut into serial sections. We have avoided this technique because it is both tedious and time-consuming. Its advantage of greater concentration of material is partly lost in examining a large number of sections. Even more important is the fact that a section of sputum can give a true picture only in those rare cases where we deal with whole pieces of neoplastic tissue. On the other hand, a seeming tissue continuity may be created from cells of different origins, consequently giving a wrong impression. Lastly, cells blocked in paraffin fail to spread out on the slide. No over-all impression of the cell in one plane

can be gained, and certain malignant changes, especially polymorphism, are much less evident.

Another way of examining tumor cells is the use of unprepared material, as was done by the older cytologists. Much is lost in this method, and only the gross abnormalities in cellular structure can be recognized. However, with the discovery of the phase-contrast microscope by Zernicke the idea is being revived (Albertini 1945 and 1947, Zinser 1949 and 1950, Doglioni 1951). Lack of adequate experience prevents us from expressing any definite opinion on this technique.

The preparation of a stained smear occurs in two stages: fixation and staining.

Fixation may be wet or dry, depending on whether the slides are immediately immersed in the fixative or first allowed to dry and then fixed. Workers employing the more histologic methods prefer wet fixation because they feel that the drying causes distortion and loss of finer structural details of the cells. At first, all sorts of fixatives used in histology were applied to cytologic preparations. Thus Bezançon and de Jong used a 1 per cent solution of chromic acid, Dudgeon applied Schaudinn's fluid, while Mathews fixed in Bouin's fluid. Eventually only two fixatives remained in vogue, viz., equal parts of 95 per cent alcohol and ether for wet fixation, usually followed by a hematoxylin stain, and methyl alcohol for dry fixation, followed by a Romanowsky stain.

We have made a comparative study of these two methods, with the aim of learning just which factors are responsible for the varying pictures encountered when different fixation techniques are applied. Our first question was whether cell distortion is actually greater after dry than after wet fixation. Obviously a large amount of dehydration also occurs with immersion in ether-alcohol. In the second place we inquired about the cause of the more detailed chromatin structure in wet-fixed smears, stained with hematoxylin, e.g., Papanicolaou's method, as compared to preparations treated according to the current Giemsa technique. Are we to attribute these differences to the fixative, to the stains, or to a possible artefact?

Human bone marrow was used as material for our investigation, as the large number of different cells in all stages of maturation made it well suited for our purposes. The smears were stained with May-Grünwald-Giemsa, hematoxylin-eosin-azure, and Papanicolaou's stain. In each case we fixed both wet and dry, and used methyl alcohol and 95 percent ethyl alcohol-ether.

Preparations stained with May-Grünwald-Giemsa and treated with wet fixation in alcohol-ether demonstrated a clearer chromatin pattern but poorer cytoplasmic detail than those which were allowed to dry and then fixed in methyl alcohol. This was probably due to differences in the fixative, since the exclusive use of ether-alcohol resulted in no distinction in cellular structure between wet- and dry-fixation smears. Also in slides stained with hematoxylin-eosin-azure, the cells looked the same whether fixed wet or dry.

Marked dissimilarity occured when hematoxylin alone was used as a nuclear stain. We employed Papanicolaou's technique and saw the following results: smears subjected to dry fixation in alcohol-ether lacked a definite chromatin structure but revealed the same even, light gray color of the nucleus seen at the edges of wet-fixed smears. (These dry up before the slide can be immersed in the fixative.) No other differences were noted.

We have therefore concluded that dry fixation prevents subsequent staining of the chromatin with hematoxylin, and that the "distortion" occurring after cells are left to dry is due to the stain rather than the fixation method. This applies only to normal cells, because dry fixation will produce disfiguration of the nucleus when the nuclear membrane has undergone pathologic changes, as in tumor cells. The nuclear content, however, retains its structure as long as stains other than hematoxylin are used. We are inclined to regard this artificial change in the form of the nucleus as an additional aid, rather than a hindrance, in detecting malignancy, since decreased tone of the nuclear membrane is one of the characteristics of malignant tumor cells.

The essential factor responsible for the finely differentiated chromatin structure in preparations stained according to Papanicolaou's method can be demonstrated by the following procedure. If a bone marrow smear is stained with a mixture of equal parts 0.1 per cent eosin and 0.1 per cent azure II and then destained for a short while in absolute alcohol, the same detailed chromatin pattern results as in hematoxylin-stained smears. It thus appears that both hematoxylin and the eosin-azure complex have a greater affinity for chromatin than for other staining parts of the nucleus. The selectivity of azure-eosin is actually slight, but becomes pronounced after destaining in alcohol. This may be explained by a varying alcohol-fastness of the different nuclear substances with respect to this dye. It should be noted that the Giemsa method also makes use of finely dispersed particles of eosin-azure in staining the nucleus.

That chromatin structures are not an artefact resulting from fixation processes has been satisfactorily shown by workers who examined unprepared cell material with the aid of phase-contrast microscopy (von Albertini 1945).

It is evident from the above that several *staining methods* are at our disposal for cytologic work; among them those which stain the nucleus with hematoxylin, and the Giemsa panoptic stain.

In our country it was Lopes Cardozo (1950 a and b) and Swieringa (1949) who introduced the latter method into the field of exfoliative cytology. Hematoxylin may well be considered a staining method derived from histology. Wandall (1944), Dudgeon and Wrigley (1935), McDonald and Woolner (1947), and Saphir (1949) have used the familiar hematoxylin and eosin technique without modification. A third procedure, which Herbut and Clerf (1946), Farber and Benioff (1948), Keuning (1950), and Dijkstra (1951) have applied to bronchial cytology, is that of Papanicolaou. Still another was developed in our laboratory, a hematoxylin-eosin-azure technique modified to cytologic work.

Papanicolaou's method is described in detail in the publication of The Vincent Memorial Hospital (1950). We were able to shorten the procedure, without any changes in the result, by omitting the regressive staining with Harris' hematoxylin and substituting a progressive method with Ehrlich's acid hematoxylin. After wet fixation in alcohol-ether and transfer via the alcohol series to distilled water, the smears are immersed in Ehrlich's hematoxylin for 3 to 10 minutes, depending on the age of the stain. The slides are then washed briefly in distilled water and subsequently held under running tap water for 5 minutes. Destaining with hydrochloric acid is thus avoided and greater uniformity of results is achieved. The remaining steps follow the original formula, with Orange-G and EA-36 as counterstains.

In the *hematoxylin-eosin method*, the slides are treated with acid hematoxylin as noted above and carried through the alcohol series up to 96 per cent alcohol. The smears are then stained in a solution of 2 per cent eosin in 96 per cent alcohol, rinsed twice in both absolute alcohol and xylol, and enclosed in Canada Balsam. Here, too, the staining process is progressive.

The *May-Grünwald-Giemsa technique* is carried out according to the usual hematologic formula. The smears are allowed to dry and fixed for 3 to 5 minutes (very fresh preparations need 5 minutes) in undiluted May-Grünwald solution. An equal amount of boiled

distilled water is added and mixed by blowing with a pipet. After standing for 1 minute, the slides are decanted. Finally, they are rinsed and covered with 15 drops of Giemsa stain in 10 cc. distilled water, which is allowed to act for 15 minutes (fresh preparations and smears of mucopurulent sputum require 20 to 25 minutes). The stain is washed off with tap water.

The formula of our hematoxylin-eosin-azure staining method is given at the end of this chapter.

We have subjected these various staining methods to a comparative study in order to determine their specificity as well as their value in routine examination.

The term specificity can be interpreted in two ways. One is the property of a dye to color certain cells in a characteristic manner. Unfortunately we know of no stain which points out qualitative differences between tumor cells and normal cells. A stain may also be specific for certain cellular substances and show them up in a preparation. The latter form of specificity is of great importance because research in the past ten years has shown significant quantitative changes in the nucleic acids of the nucleus and cytoplasm of tumor cells (Caspersson, 1950; Koller, 1953; Stowell, 1942 and 1945; Stowell and Cooper, 1945).

There are a number of staining reactions for nucleic acids. Desoxyribonucleic acid (DNA) in the nucleus is stained red in the *Feulgen reaction,* when previously depolymerized and hydrolyzed. The *methyl green-pyronine stain* of Unna-Pappenheim gives polymerized DNA a green color (Stowell, 1945a, and Pollister and Leuchtenberger, 1949).

We may now inquire to what extent the Giemsa or the Papanicolaou techniques are specific for nucleic acids. Stowell (1945a) demonstrated that cells are lightly stained with hematoxylin even when a negative Feulgen reaction in control smears has shown complete hydrolysis of DNA. He attributed the remaining color to incompletely hydrolyzed, protein-like cell elements. We have applied Stowell's experiment to the stains of Papanicolaou and May-Grünwald-Giemsa. Both of these techniques are known to demonstrate a hyperchromatism of the nucleus, cytoplasm, and sometimes nucleolus in cells with a raised nucleic acid content (Keuning, 1951). By no means, however, is the conclusion justified that these methods demonstrate only a nucleic acid increase, to the exclusion of any other substance. It should furthermore be understood that "chromatin" is purely a morphologic concept; nucleic acids are associated with the chromatin linin network

rather than constituting the chromatin.

Before the relative values of the two techniques are discussed, a brief description is given of their results.

In Papanicolaou's method the chromatin framework is stained bluish gray, and the nucleoli are usually indistinguishable from chromatin condensations. The cytoplasm varies from bluish gray to green and to orange, depending on the grade of basophilism or oxyphilism.

The May-Grünwald-Giemsa method gives the nuclear content a purplish color, but apparently stains more than the chromatin. The result is that the nucleus presents a rather compact appearance. The nucleoli are colored blue when they are active. A basophilic cytoplasm stains blue, but with diminishing basophilism and increasing oxyphilism, it becomes light blue and finally orange-pink, with gray as an intermediate, polychromatic color.

A series of contact smears of a partly anaplastic carcinoma served as material for our investigation. Its cells exhibited a very active structure of hyperchromic nuclei and numerous darkly-staining nucleoli. The hyperbasophilic cytoplasm stained bluish gray and dark blue with Papanicolaou and May-Grünwald-Giemsa respectively.

Several series of preparations were hydrolyzed for different time periods in a 1 N solution of hydrochloric acid at 50° C., and each series was then treated according to the following methods: (1) Feulgen reaction (Stowell 1945a); (2) Unna-Pappenheim (methyl green-pyronine); (3) May-Grünwald-Giemsa (M.G.); (4) Papanicolaou.

In preparations that were not hydrolyzed the Feulgen reaction was negative, as would be expected. Methyl green-pyronine stained the nuclear chromatin green. Papanicolaou and M.G. stained smears have already been described above.

Preparations hydrolyzed for 20 minutes and then subjected to Feulgen's reagent yielded a strongly positive reaction for DNA. The red stain was so localized that the chromatin of the nucleus displayed the same pattern as in cells treated with hematoxylin without preceding hydrolysis. The latter observation has been confirmed in our bone marrow preparations on numerous occasions. With methyl green-pyronine no green color could be observed in the nucleus, suggesting that all the polymerized DNA had been converted into the depolymerized state in the 20 minute period. The M.G. slides exhibited intensely stained red-purple nuclei, but differed essentially from unhydrolyzed smears in that the chromatin pattern was now very pronounced. The nucleus thus had the same open structure seen

in ordinary hematoxylin smears. The originally deep blue nucleoli could not be distinguished and the cytoplasm, previously strongly basophilic, now stained light bluish gray. The Papanicolaou smears displayed a more lightly colored nucleus, a much thinner chromatin mesh, red nucleoli, and a greenish gray cytoplasm.

Hydrolysis of a third series for 90 minutes yielded the following results: the Feulgen reaction was negative, pointing to complete decomposition of DNA. The Unna-Pappenheim was once again negative. In the M.G. preparations we saw mere shadows of cells, which stained light grayish violet, and had pink spheres at the sites of the nucleoli. The Papanicolaou smears likewise displayed only cell ghosts colored light gray, with pink spots representing the places of the nucleoli.

If we consider the staining substances in a cell as falling into four groups, namely: (1) polymerized DNA, (2) slightly or unpolymerized DNA, (3) ribonucleic acid (RNA), and (4) other substances, among them proteins such as the acidophilic histones, then certain conclusions will appear from the above experiments.

Neither the M.G. nor the Papanicolaou technique is specific for nucleic acids in the sense that exclusively these substances are demonstrated; cells were colored slightly after complete breakdown of the nucleic acids had taken place. The remaining color must of necessity be due to substances in the last category.

After 20 minutes of hydrolysis we observed a distinct change in color and staining intensity of the cytoplasm and nucleoli. In addition, the M.G. smears showed an increase in definition of the chromatin network. It is especially the color change of the nucleoli, at first concealed in the M.G. series by the intensely red-stained chromatin, but visible after 90 minutes of hydrolysis, that speaks for a complete decomposition of the unpolymerized, or only slightly polymerized RNA. This argument is further supported by the fact that the color of the nucleoli in the Papanicolaou smears displayed no further alteration in the 20 to 90 minute period of hydrolysis.

It may be assumed that unpolymerized DNA is also decomposed after 20 minutes. The methyl green reaction of the nucleus became negative in that time. Consequently it seems likely that in these 20 minute series, depolymerized (originally highly polymerized) DNA is associated with the chromatin structure. It will be recalled that the chromatin here was so well defined in the M.G. preparations, but only lightly colored in the Papanicolaou smears.

If we assume that the M.G. stain has about the same affinity for polymerized as for unpolymerized nucleic acids, whereas hematoxylin is bound more strongly to the polymerized structures, then the above findings are readily explained.

In unhydrolyzed preparations the M.G. staining method colors high-molecular and low-molecular DNA with more or less equal intensity, with the result that the nucleus has a dense appearance and the chromatin pattern is somewhat concealed. Hematoxylin, on the other hand, stains especially the high-molecular nucleic acids, so that the chromatin structure stands out clearly. After 20 minutes of hydrolysis, those depolymerized nucleic acids derived from high-molecular DNA stain intensely with M.G., whereas hematoxylin now imparts a weaker color to the nucleus.

Certain advantages of the M.G. stain over hematoxylin now become apparent. Considering that it demonstrates unpolymerized and polymerized nucleic acids equally well, it is the better stain for RNA of the cytoplasm and the nucleolus. This is undoubtedly one of the reasons why Giemsa reveals greater cytoplasmic detail and shows the nucleoli to full advantage. A second important point is that DNA and RNA are differentially stained. RNA becomes blue, as suggested by the color change of the nucleoli from blue to pink during hydrolysis, while DNA becomes purplish red. With hematoxylin this distinction is not possible, both substances staining blue-gray. As hematoxylin has but slight affinity for low-molecular nucleic acids, it demonstrates cytoplasmic RNA only when the latter is present in high concentrations. In our preparations of anaplastic carcinoma cells it did stain the cytoplasm light blue-gray, but in many cases hematoxylin will be concealed by the counterstain employed (e.g., Papanicolaou's method).

Besides being indistinguishable from the DNA associated with the chromatin, the nucleolar RNA stains with but little intensity in hematoxylin methods. If we allow hematoxylin to act for only a short time, and then stain with eosin, the nucleoli will be colored red in a completely nonspecific manner.

Table III presents a summary of the differences between the two stains discussed above.

TABLE III

The colors taken on by the various cyptoplasmic and nuclear constituents treated with May-Grünwald-Giemsa and with hematoxylin, before and after fractional hydrolysis in 1 N HC1 at 50° C.

	M.G. stain	Hematoxylin
Polymerized DNA	purplish red	blue-gray
Unpolymerized DNA	purplish red	almost unstained
Nucleolar RNA	blue	light blue-gray
Cytoplasmic RNA	blue	light blue-gray
Proteins and other staining substances of the nucleoli, after 90 minutes of hydrolysis	pink	pink
Proteins and other staining substances of the cytoplasm, after 90 minutes of hydrolysis	light gray-violet	light gray

If one is to estimate the value of a staining method for routine examination, expecting it to yield optimal conditions for the clinical-cytologic diagnosis of cancer, one must bear in mind the requirements which such a method should fulfil. In our opinion the procedure must be as simple as possible and not time-consuming; it should be reliable in giving persistently the same results; lastly, it must produce a detailed cell picture. The first two items, when considered from a clinical point of view, are quite obvious. For the last we offer the following explanation: in arriving at the proper diagnosis of a malignant process it is essential to determine whether a given cell is atypical, and a group of cells exhibits polymorphism in the broadest sense of that word. Evidently, the more detail a cellular structure

reveals, the greater is the number of points to which we can refer in classifying a given cell as typical or atypical, and the more items of comparison are at our disposal to establish any degree of polymorphism. Moreover, certain criteria of malignancy are seen to better advantage, as is the case in nucleolar pathology.

Inasmuch as the May-Grünwald-Giemsa stain meets the above requirements, we have chosen it for routine examination in preference to other methods. Lopes Cardozo has pointed out the additional value of the M.G. stain in facilitating comparison with other branches of clinical cytology. For example, tumor cells from a lymph gland punctate may be compared with those found in the sputum of the same patient.

We have already indicated above that Papanicolaou's technique fails to bring out all the definition in cell structure found in Giemsa preparations. If a hematoxylin method is still desired, in order to gain a better basis of comparison with histologic sections, we prefer the usual hematoxylin-eosin stain because it is more economical and timesaving. Moreover, it is hematoxylin which is responsible for the chromatin pattern in Papanicolaou smears. As far as the transparent cytoplasmic stain with Orange-G, Light Green, Bismarck Brown, and eosin is concerned, we are inclined to regard it as superfluous. Only a slight differentiation between basophilism and oxyphilism is gained, without any addition in structural detail. The establishing of basophilism is only significant when it points toward an increase of cytoplasmic nucleic acid, and that is brought out by hematoxylin. Tumor cells which demonstrate an appreciable degree of cytoplasmic basophilism in the Giemsa smear actually have a light blue-gray cytoplasm in Papanicolaou preparations.

Keratin shows up just as well with eosin as with Orange-G. Keratinization is demonstrated with great clarity in Giemsa smears, as will appear later on.

It remains to be mentioned that we have modified the Maximow staining technique (Bloom) with the aim of effecting a better chromatin structure with a minimum loss of cytoplasmic detail. This method presents some of the features of Giemsa as well as of hematoxylin. Hematoxylin probably assumes here the role of a mordant with respect to the azure-eosin mixture, and a well-defined chromatin pattern is the result.

Our technique is as follows: (1) Wet fixation in equal parts 96 per cent alcohol and ether for 30 minutes. (2) Ehriich's hematoxylin

for ¼ to 2 minutes, depending on age of the solution. The smear should now be but lightly stained. (3) Wash in distilled water for 10 minutes. (4) Stain in the azure-eosin mixture for 15 minutes. The mixture is prepared just before use by adding equal parts of 0.1 per cent eosin and 0.1 per cent azure II solutions. (5) Destain in absolute alcohol for a few seconds. 6) Rinse in absolute alcohol. (7) Rinse twice in xylol. (8) Enclose in Canada Balsam.

The result is a blue-staining chromatin structure of extremely sharp definition, dark violet to black nucleoli, and a cytoplasm that varies between pink and blue-gray.

However, the stain is more transparent than panoptic, and somewhat unstable due to the destaining in absolute alcohol. Therefore the May-Grünwald-Giemsa procedure is still better suited for routine laboratory examination.

The specific staining methods with Feulgen's reagent and methyl green are not appropriate for cytologic work, because the specificity they offer is accompanied by a lack of structural detail. They should be reserved for qualitative and quantitative analysis in cytochemistry.

CHAPTER III

MORPHOLOGIC CRITERIA OF MALIGNANCY

Although numerous workers have summarized morphologic characteristics of malignancy we have never found a systematic survey of the subject. We therefore decided to consider these characteristics, and especially to inquire as to what extent cytomorphologic criteria of malignancy really exist. We have furthermore attempted, as Koller (1943), Stowell (1945), and Keuning (1951) have done, to establish a relationship between structural abnormalities and the physiology of normal and unrestrained cell growth.

Of special significance in the latter case is the function of the nucleoproteins, which Caspersson (1950), Koller (1943), Stowell (942), and Stowell and Cooper (1945) have studied. Their experiments indicate that an unequal distribution of DNA among the cells, rather than an absolute increase in the amount of DNA, characterizes malignant growth. As a matter of fact, Stowell noted a decrease in the DNA content of certain carcinomas. Ultimately it is this irregular distribution which accounts for the most important mark of identification of tumor cells: polymorphism in its broadest sense.

The increase of DNA in a certain percentage of cells is attended with a series of events such as accelerated mitotic division, a shortened interphase, and a larger production of proteins, especially histones and globulines, during particular stages of growth. Moreover there is stronger adhesion among the chromosomes due to the greater viscosity of the increased polymerized DNA. This stickiness seems to promote the morbid growth and to maintain it autocatalytically by starting a vicious circle. The chromosomes and the nucleic acids are thus divided unequally during mitosis, whereby certain daughter cells with too much DNA are continually formed. Adhesion is also responsible for polyploid cells and abnormal mitotic figures. If we imagine that the large amounts of DNA in a cell ultimately impede mitosis, then we have a ready explanation for the occurrence of amitotic division and giant cells.

The irregular distribution of DNA manifests itself in anisocytosis and anisonucleosis, as well as in varying degrees of hyperchromatism or lack of chromatin. Aside from giving rise to a polyploid number of chromosomes, large quantities of polymerized DNA lead to the formation of an irregular, coarsely-fragmented chromatin structure in the interphase. Hyperbasophilism of the cytoplasm and, at times, the presence of numerous darkly-staining nucleoli are signs of augmented protein synthesis and increased RNA production in certain growth phases.

We may regard a malignant process as limited in its early stages to cells of one degree of differentiation. If this assumption is correct, then the abnormal cell divison will finally stop all growth at that stage of development, and the process will have to revert to one of lesser differentiation. The trend toward anaplasia thus seems to be inherent in malignant growth.

Degeneration, in the sense of necrobiosis, is often met with in cancer cytology, just as it is in histologic sections of tumors. Its distinctive marks will be summarized below among the criteria of malignancy. It must be clearly understood that degeneration alone can never be used as positive evidence of malignancy, since it accompanies conditions other than malignant growth.

The problem of differentiating specific from nonspecific criteria becomes even greater when a process of necrobiosis, caused by traumatic, toxic, infectious, or other agents, is accompanied or followed by *regeneration, hyperplasia,* and possibly *metaplasia.* The fact that the surroundings of a neoplasm may exhibit regeneration adds to the confusion.

Although the postulate that there are no fundamental qualitative differences between the morphology of normal and tumor cells is basically true, we need not deny the existence of specific structural criteria of malignancy. Where two conditions differ only in certain quantitative respects from each other, so that a gradual transition can be observed, there will be a question of ambiguity and lack of definition only in that area of transition. At opposite extremes of the dividing line such pronounced differences may exist that the distinction becomes almost qualitative. Consider the question of an adequate physiologic definition of life. The difference between living and dead matter is striking until we reach the sphere of virology, where all distinctions seem to be lost.

In dealing with cancer we usually meet situations far removed from the boundary of malignant and non-malignant growth, and the

search for specific marks of differentiation is well justified. By focusing our attention on the points of differences which follow, we regard it possible to distinguish cells of regenerating tissue from those of a malignant tumor. (1) Cells of regenerating tissue do not exhibit any atypia (metaplastic epithelium is an exception to this rule). Polymorphism may exist to a considerable degree, but all structures and substances met with in the normal cell are also encountered in the regenerating cell (e.g., cilia and an acidophilic area in polymorphic, regenerating ciliated epithelium). (2) It follows that anaplasia never occurs in regenerating tissue to any great extent; some well differentiated cells always appear in the preparation. This tendency to differentiate is also evident in metaplastic epithelium, as well as in benign tumors like the bronchial adenoma (Figs. 7 and 10). (3) The nuclear-cytoplasmic ratio always corresponds to the degree of maturation of the cell. (4) The nucleolar-nuclear ratio stays within normal limits. (5) The nuclear membrane, like the nucleus itself, is evenly shaped (compare Figs. 7 and 10 with Figs. 13 and 15). (6) The cytoplasm is sharply delineated from its surroundings (compare Figs. 7 and 28). (7) The nucleus and the cytoplasm are at the same stage of development, so that a metaplastic epithelial cell with a young nucleus, for example, will not display keratinization. This rule applies in all of cytology (Bessis, 1948). (8) Abnormal mitotic figures are not encountered. (9) Nonmalignant epithelial structures lack the pronounced tendency to exfoliate, unless necrobiosis complicates the process. Signs of necrosis or purulent inflammation in a smear thus indicate caution in arriving at a quick diagnosis. This is especially significant in view of the many reports in literature of incorrect diagnoses of carcinoma in patients with bronchiectasis and pulmonary abscess. In the sputum of these patients mucopurulent and necrotic pieces of tissue were found to contain cells from metaplastic epithelium which were mistaken for malignant elements (Papanicolaou and Cromwell, 1949; Herbut and Clerf, 1946; McDonald and Woolner, 1947). The metaplastic cells of bronchial epithelium are described in Chapter IV.

The following is a systematic review of the cytologic criteria of malignancy and the morphologic changes which may appear as a result of a cancerous process. We will discuss these under three categories: characteristics of individual cells, characteristics of cell groups, and general characteristics.

CHARACTERISTICS OF INDIVIDUAL CELLS

The nucleus.

The most essential change is the increased nuclear-cytoplasmic ratio in cells of all degrees of maturation. The shape of the nucleus may deviate strongly from the normal, which is partly due to the irregular structure of the nuclear membrane. The latter is well illustrated in a dry fixation smear. The nucleus thus gets a very bumpy outline, and, in more extreme cases, is wedged in or lobulated (Figs. 15, 18, 30, 32, 52, 57).

Many abnormalities of the chromatin structure can be attributed to degeneration, although the presence of necrobiosis may only be inferred from fresh material. Among the morphologic signs of this nonspecific phenomenon are pyknosis (Figs. 14, 17), karyorrhexis (Fig. 31), hyperchromatism of the nuclear frame and nuclear border (Fig. 32), and nuclear vacuolation (Fig. 57). A specific sign of malignancy is hyperchromatism of the nucleus, which must be differentiated from pyknosis and hyperchromatism of the nuclear frame and border. Pyknosis, as Leuchtenberger (1950) and Korson (1951) have demonstrated, is attended with a gradual loss of nuclear DNA. Condensation of the remaining chromatin causes the dark color, and is accompanied here by a reduced nuclear-cytoplasmic ratio. In true hyperchromatism the darkly-staining nucleus retains an unimpaired chromatin structure. Moreover, there is no "hyperchromatism" of its frame and border, and the nuclear-cytoplasmic ratio is normal or even increased. (Figs. 15, 24).

Likewise specific for malignant tumor cells and of great importance is an irregular chromatin structure accompanying the hyperchromatism. In contrast to the structureless mass of chromatin seen above, we have here a very coarsely reticulated chromatin network in which fragments of all sizes can be found (Figs. 14, 15, 24, 50).

Multinuclear cells may occur, especially in polymorphic squamous cell carcinomas. However, the decision of malignancy must be

restricted to structures where the various nuclei differ considerably from one another in size, shape, and color intensity (Figs. 27, 28, 29).

The nucleoli.

There is in the first place an increase in the nucleolar-nuclear ratio (Fig. 58). Multiple nucleoli (Fig. 57) are often seen, although this is by no means as universal as literature might suggest. Some carcinomas exhibit little if any nucleolar pathology, others only in certain stages (particularly the anaplastic stage). Of significance is the unequal size of nucleoli within one nucleus or in different nuclei.

Hyperbasophilism of the nucleolus may be an expression of morbid synthetic activity, although this may have an entirely different meaning in cells undergoing necrobiosis or autolysis. Finally it should be borne in mind that nucleoli may be chromophobe. This occurs especially in rapid-growing neoplasms, where only light, clear areas, often surrounded by chromatin condensations, are visible (Fig. 51).

The cytoplasm.

A poorly defined cytoplasmic border is unequivocal evidence of anaplasia. It ultimately results in complete loss of the cytoplasm, leaving only bare nuclei (Figs. 52, 55, 57). Hyperbasophilism has already been discussed in the beginning of this chapter.

Various stages of keratinization can be observed in smears of squamous cell carcinomas (Figs. 13, 15, 17). Under certain conditions the normal squamous epithelium of the mouth and pharynx, or metaplastic epithelium of the bronchi may also become cornified, so that keratinization is a reliable indication of cancer only when the cells show other signs of malignancy.

Phagocytosis of leukocytes, erythrocytes, or nuclear fragments by cells of epithelial origin strongly suggests malignant degeneration of those cells (Fig. 19). Of the lung tumors it is especially the squamous cell carcinoma which exhibits this property. One aspect of it is autophagocytosis, whereby a more or less digested cancer cell is found enclosed within the cytoplasm of another (Figs. 21, 23, 24). This may result in a form which the histologists have labeled as "bird's-eye cell" (Fig. 22). Some of these cells seem in turn to be phagocytosed by others, the nucleus of the parent cell being pushed to the periphery and the cytoplasm reduced to a thin rim. If we imagine that this process could repeat several times, then it seems not unlikely that the bird's-eye cell represents the beginning of epithelial pearl formation. (Fig. 20).

Vacuolation may be part of a process of degeneration (Fig. 18), but brought out in May-Grünwald-Giemsa smears; namely, a peculiar, polychromatic ground substance, studded with fine, red, azurophilic

in the absence of other signs of necrobiosis points toward secretory activity. This is very likely the case in cells containing many small or a few large vacuoles and a flattened nucleus at the periphery. If a cell with malignant characteristics presents such a picture it is probably derived from a mucigenous adenocarcinoma (Figs. 41, 42, 44).

In most carcinomas, with the exception of the small cell type, there is an increase in the average size of the cells. *Uninuclear or multinuclear giant cells* are a rather frequent occurrence in smears of squamous cell and adenocarcinomas, and can easily be spotted at low power magnifications (Figs. 24, 25, 26, 29).

CHARACTERISTICS OF CELL GROUPS

Large and irregularly bounded clusters of cells in a smear of sputum or bronchial secretions are in themselves suggestive of carcinoma (Lopes Cardozo, 1950a), especially when these cells are atypical (Fig. 12).

Polymorphism has already been discussed. The term, as used here, includes variety not only in shape but also in size and staining intensity of all the organs and constituents of the cell. In addition it refers to variety among cells and cell clusters (Figs. 11, 25).

Mitoses are much more sparse in cytologic preparations than in sections of the same tumor, due to the great difference in cell density. Their presence in a smear indicates a rapid growth. If atypical mitoses are also seen, no doubt should remain that a malignant process is at hand. Abnormal mitotic figures may include any of the following: a polyploid number of chromosomes (Fig. 34); irregular size and distribution of the chromosomes over the surface of the cell; clumping together of part of the chromatin material (Fig. 41); large, irregular astral figures (Fig. 34); triple and multiple spindle formation. The latter is very rare and we have observed it only once.

In conclusion, atypical mitosis can be judged by the *resulting product*, which may be unequal size of daughter nuclei in an otherwise undivided cytoplasm (Fig. 27), or two nuclei connected to each other by a thin chromatin filament (Fig. 35).

GENERAL CHARACTERISTICS

Necrosis.

In addition to what has been said about the appearance of the necrobiotic cell there is another manifestation of necrosis which is

spots (Fig. 36). Further evidence of necrosis are alveolar cells and other macrophages filled with lipoid vacuoles (lipophages).

Hemorrhage.

Erythrocytes may attest to an artificial lesion, such as occurs during bronchoscopy. If the hemorrhage is not a recent one, the erythrocytes are disintegrated and anisocytotic, often staining blue with Giemsa. Siderophages will also be present; these are described in the following chapter

Inflammation.

Evidence of inflammation is frequently noticed in smears of tumor cells. One sees large numbers of polymorphonuclear leukocytes in acute inflammation, and lymphocytes, plasma cells, and histiocytes in more chronic processes. In our work with cancerous material we often noted quite a large number of eosinophilic granulocytes and, to a lesser extent, mast cells.

Polymorphism and metaplasia.

These are changes often met with in the bronchial epithelium in the vicinity of a tumor. They are considered in the next chapter.

It is obvious that all of these characteristics are seldom found in one preparation. One cell certainly never exhibits them all, which means that malignancy cannot be diagnosed with any reliability on a basis of abnormalities seen in a single cell.

Histologists have leveled strong criticism at the cytologic method of cancer diagnosis, because infiltration, an important property of malignant tumors, is missed in this type of examination. When one considers, however, the rich variety of cytologic criteria, some inaccessible to the histologist, others seen to much better advantage in a smear, the inability to determine infiltration does not seem a serious loss to us. This argument is all the more valid in cases where the section shows no signs of infiltration, and the histologist is dependent on a cytologic interpretation to aid him in his diagnosis.

CHAPTER IV

NORMAL CELL MORPHOLOGY AND SOME NONMALIGNANT CYTOLOGIC PICTURES OF THE RESPIRATORY TRACT

In this chapter and the one to follow, we will attempt a description of the cell morphology of sputum, endobronchial material, and lung punctates in smears stained by the May-Grünwald-Giemsa method. The order of our account corresponds to the anatomic and histologic origin of the various cells. To avoid needless repetition it seems best first to outline the essential differences in the morphologic aspects of the various material used.

The term "sputum" must be qualified because pharyngeal sputum contains only elements derived from the mouth and pharynx. Bronchus and lung sputum, on the other hand, shows us cells from the whole tracheobronchial tree and the alveoli. Obviously it is only the latter from which information concerning a bronchial or pulmonary process can be obtained. Furthermore, any bronchial or pulmonary exudation is usually part of a pathologic condition, so that a "normal morphology of sputum" is a rather fallacious concept. Sputum differs in this respect from material acquired by lung puncture, cotton swab, or biopsy, where normal, healthy cytologic pictures can be described. It should also be borne in mind that normal epithelial cells do not exfoliate; hence the majority of cells in a sputum preparation will be of mesenchymal origin. The few epithelial cells that are encountered show signs of degeneration. Carcinoma cells, which do exfoliate, may also appear in the sputum in a degenerated form, having undergone autolysis during their stay in the bronchial lumen.

The bronchial secretions are apt to contain cells with little or no degeneration, as the introduction of instruments into the bronchi causes artificial exfoliation. It is only in contact smears of portions of biopsy tissue or lung punctates that we encounter the cells in a more natural state. On very rare occasions, the cytologic picture is complicated by cells from another organ system, which have reached the respiratory tract by means of abnormal connections, e.g., an esophagotracheal fistula.

The normal *epithelium of the mouth and pharynx* is of the stratified squamous variety, of which the superficial layers constantly degenerate and desquamate. The desquamated cells are therefore found in every sample of sputum, and may at times be seen in bronchial secretions, having been transported there during the introduction of a tube or bronchoscope. The more basal cells are seldom encountered in the sputum, except where trauma, chronic cough, ulceration, etc., cause great exfoliation.

The superficial epithelial cells (Fig. 1), are conspicuous for their large size, rectangular or polygonal shape, and very small nuclear-cytoplasmic ratio. Their round or oval nuclei usually display pyknosis or chromatolysis. Nucleoli are nearly always absent. Sometimes the cytoplasm is completely degenerated, and nothing is left but accumulations of bare nuclei. Where the cytoplasm is still fairly well preserved it stains light blue to gray, rarely pink, and has a fine, granular appearance. Keratohyalin is never encountered, and keratinization is not seen to the same extent as in cornifying squamous cell carcinoma. (For a description of the various stages of keratinization, see the section on keratinizing squamous cell carcinoma.) Bluish purple-staining microorganisms on the surface or inside of these cells indicate their oropharyngeal origin.

The cells derived from the intermediate and basal layers (Fig. 2), are round or oval, their size varying according to the layer of origin. The relation of nucleus to cytoplasm is greater than in cells from the superficial layers, but never attains the proportions seen in metaplastic bronchial epithelium. The round nuclei have even borders, and the chromatin structure, if not pyknotic, is finely granulated. One or two small, light-blue nucleoli may sometimes be detected. The cytoplasm is sharply delineated and stains light-blue or gray. We have never observed keratohyalin in these cells, although traces of keratin were sometimes seen. The basal cells are a rare occurrence in sputum. They are small, often angular cells with round, dark nuclei and a dark blue cytoplasm.

The nasopharynx, larynx, trachea, and bronchi are lined with a pseudostratified columnar ciliated epithelium (except the vocal cords) whose layers become fewer and the cells lower the more peripheral we go. Finally, in the terminal bronchioles, there is a simple cuboidal epithelium. The cells which present themselves in the sputum are very few and usually more or less degenerated.

Cells derived from the superficial layer (Fig. 3), are commonly seen in small, compact groups. Their shape is cuboidal or columnar, with the basal extremities often pointed. The oval or spindle-shaped nuclei

are situated in the basal part of the cell and have regular boundaries. One or two small, light blue nucleoli are often seen within the fine, compact meshes of the chromatin. The cytoplasm is colored blue in the basal part, but turns to a lighter shade in the apical region, gradually becoming pink with fine, red granules at the very top. What was originally the lumen end of the cytoplasm terminates abruptly and makes way for a faintly staining margin which contains at its outer part a row of red basal corpuscles. Each of the latter lends origin to a cilium. Occasionally one encounters secretory vacuoles, which are situated at the lumen side of the nucleus. They may coalesce to form such large vesicles that the nucleus is flattened out at the bottom of the cell (goblet cell).

When ciliated epithelial cells degenerate, they display the usual characteristics of necrobiosis and, in addition, lose their cilia (Hers and Mulder, 1951). Moreover, the acidophilic granules referred to above fuse to form a spherical conglomeration at the apical pole of the cell, while the nucleoli appear much darker. The picture may be complicated by a complete loss of cytoplasm, only the form and structure of the consolidated nuclei hinting at the origin of the cells.

Regenerating ciliated epithelium presents a rather polymorphic collection of sometimes very large cells, which frequently have two or more nuclei but otherwise look normal. The cytoplasm and nucleoli are strongly basophilic and there is evidence of secretory activity.

The basal cells from the epithelium of the nasopharynx and respiratory tract, like those of the squamous epithelium, are rarely encountered in sputum. They are oval or pear-shaped cells with completely basophilic cytoplasm and a nuclear structure identical with that of the ciliated cells.

The question whether an epithelial lining of *the alveoli* of the lung actually exists still remains unsettled. The origin of the supposed cells is likewise dubious. Epithelization of the alveoli and single alveolar cells in the lumen is a picture which can, as a matter of fact, be observed in several chronic pulmonary diseases. Whether these cells are mesenchymal derivatives originating from the septal stroma (Bos, 1951), or represent a metaplasia or hyperplasia of the hypothetical alveolar epithelial cells is still disputed.

This problem is of significance in connection with the cytologic structure of tumors arising from the alveoli. We will attempt to show later on that under pathologic circumstances the origin of the alveolar cells may be a multiple one: (1) normal or metaplastic bronchial epithelium may grow down and line the alveoli; or (2) the cells may

be normal, possibly hyperplastic alveolar cells. It is our conviction that mesenchyme is the more likely origin of the alveolar cells, since they strongly resemble histiocytes, not only in appearance but also in their ability to phagocytose. Their presence in large numbers in lung sputum, bronchial secretions, and lung punctates, without any indication of degenerative change, is further evidence of their mesenchymal origin. Healthy epithelial cells do not exfoliate to such an extent.

The alveolar cells (Figs. 4 and 5), constitute a polymorphic group and reflect a definite series of developmental stages. The nuclear-cytoplasmic ratio is quite large in the young cells, but is considerably reduced in the older ones. The nucleus, whose size is roughly twice that of a neutrophil granulocyte, has an eccentric location. In immature cells it is round or oval, while in the more mature cells it is bean-, rod-, or shoe-shaped and may sometimes become lobulated and segmented. The chromatin is delicate and loosely meshed. As a rule, the young forms contain one or two small basophilic nucleoli. The cytoplasm is strongly basophilic in immature forms but gets a polychromatic, gray aspect in more developed cells. Finely dispersed azure granules are sometimes recognized in the latter.

Very characteristic of the alveolar cells is their ability to phagocytose pigments, a property which Hampeln (1897) already saw as an important point in differentiating them from carcinoma cells. The ingested material may be carbon, which usually appears as minute gray or black particles within the cytoplasm of the so-called "dust cells" (Fig. 4). Where small, multiple hemorrhages have occurred in the lung, hemosiderin pigment is phagocytosed, and is visible in "siderophages" as dark, blue-green granules and fragments much coarser than carbon particles. These siderophages (Fig. 5), once called "heart failure cells", occur not only in chronic pulmonary congestion but wherever there is bleeding into lung tissue, as is often the case in bronchogenic carcinoma. A third type of macrophage that develops from alveolar cells is the "lipophage", observed in connection with necrosis of lung tissue or exudation into the alveoli. Its cytoplasm contains numerous lipoid vacuoles which give the cell a foamy appearance. Phagocytosis of nuclear fragments of epithelial cells or leukocytes is also found under such circumstances. After the introduction of lipiodol into the bronchial tree, the alveolar cells are frequently studded with large, pinkish-red granules, presumably containing a product in the breakdown of lipiodol.

Whenever large amounts of pigment or foreign substances accumulate, and their removal is impeded, the alveolar cells react

to this condition by forming foreign body giant cells. Hers (1951) has stressed the importance of distinguishing these from a different type, the metaplastic giant cell which is discussed below. Alveolar giant cells (Fig. 6), may contain as many as 15 nuclei, whose structure, shape, and peripheral arrangement resemble that of normal alveolar cells. The cytoplasm is also lightly basophilic or polychromatic. In the latter case it contains fine azure granules. These cells, in contrast to metaplastic giant cells, ingest pigments, lipoids, and cellular fragments.

In a number of cases of pulmonary fibrosis and once in a case of metastatic liver carcinoma in the lung, we encountered alveolar cells of a decidedly different nature. These cells had a rather hyperplastic appearance and showed marked anisocytosis. The eccentric nuclei were for the most part small. They had a coarsely reticulated chromatin structure which otherwise was the same as in normal alveolar cells. Occasionally a large, round nucleus with a dense chromatin mesh presented itself. The relationship of nucleus to cytoplasm was normal everywhere. The latter was strongly basophilic and sharply delineated, in contrast to that of the usual alveolar cells. The intensity of the stain increased toward the periphery of the cytoplasm, and a border appeared now and then, which resembled a prekeratin ring. All of the above characteristics gave these cells the semblance of a benign version of the alveolar cell carcinoma described in Chapter V. Possibly we dealt with *hyperplastic*, or even *metaplastic*, alveolar cells which originally lined the alveoli.

The supporting structures of the lung are not met with in sputum except where gross destruction of lung tissue has taken place. Also in contact smears of biopsies they are rarely seen because only the mucous membrane is removed while the more firmly anchored connective tissue is not.

When they do appear in the Giemsa preparation elastic fibers stain purple-red. Cartilage is represented by bright red, structureless particles which have an irregular shape. Young connective tissue elements, in the form of reticular cells and fibroblasts, were seen once in a biopsy contact smear of a nonspecific granuloma. Collagenous or muscle fibers were never observed in our material.

Exudation into the bronchi and alveoli is recognized during its acute phases by the appearance of large numbers of polymorphonuclear leukocytes in the sputum. If allergy is a contributing factor in the exudation, eosinophils will be seen to a greater or lesser extent. The neutrophil and eosinophil granules stain almost identically in the

May-Grünwald-Giemsa preparation, but the latter are recognized by their larger size. In subacute and chronic stages one sees plasma cells, lymphocytes, and histiocytes. It should be remembered, however, that accumulations of lymphocytes are part of the normal aspect of the bronchial mucosa. Histiocytes, which we will not describe here in any detail, are smaller than the alveolar cells and look exactly like the monocytes from the peripheral blood.

The following is an account of our findings in three nonmalignant cytologic pictures of the lung and tracheobronchial tree.

METAPLASIA OF THE BRONCHIAL EPITHELIUM

Earlier in this chapter we have outlined the cell morphology of regenerating ciliated epithelium. It frequently happens, however, that the damaged epithelium no longer regenerates. In its place, a nonspecific stratified squamous epithelium is formed, which may be continued into the alveoli. This atypical regeneration, incorrectly called metaplasia, occurs especially in chronic inflammation of the bronchi and bronchioles, notably, bronchiectasis (Mulder and Hers, 1954) and pulmonary abscess. It is seen moreover in tuberculous bronchitis, infections with epitheliotropic viruses (Hers and Mulder, 1951), in the vicinity of a carcinoma, at the site of a foreign body (Lignac, 1939), and in certain forms of pulmonary fibrosis. Several of the above-mentioned diseases yielded us sputum, contact smears, or bronchial secretions in which atypical epithelial cells, usually in compact groups, appeared.

The cells of the metaplastic bronchial epithelium (Fig. 7), may be round, somewhat pear-shaped, or polygonal. The nuclear-cytoplasmic ratio is considerably large in the undifferentiated cells. The nuclei are usually round with a slightly smaller curvature on one side than on the other. In the young, less differentiated cells the chromatin net is dense and contains one to four small, light blue nucleoli. In differentiated cells the chromatin structure is homogeneous and somewhat pyknotic. The sharply demarcated cytoplasm is strongly basophilic and bears a delicate fibrous structure. We never saw any suggestion of keratinization. The cells in our material were not differentiated to a greater extent than the two groups described above. Any further differentiation would probably make them indistinguishable from superficial cells of the oral and pharyngeal epithelium.

In histologic sections, Hers (1951) was able to demonstrate syncytial masses of metaplastic epithelium, which grew into the bronchial lumen and became separated. The resulting structure is a metaplastic

giant cell, which appeared quite often in our smears (Fig. 8). Such cells may contain as many as 50 nuclei, whose individual structures do not differ from those in metaplastic epithelial cells. The nuclei tend to concentrate in the center, rather than at the periphery as in alveolar giant cells. The cytoplasm stains deeply blue in Giemsa smears, and is much darker than in alveolar giant cells. No ingested particles or lipoid vacuoles were ever found.

PULMONARY TUBERCULOSIS

Wandall (1944) reported the frequent occurrence of certain abnormal cells in the sputum of tuberculosis patients, and regarded these as specific for this condition. In general we were unable to confirm these findings. It seemed to us that the cytologic abnormalities seen in sputum and endobronchial material of such patients often were rather nonspecific. The only cell type that we observed with moderate frequency was that of metaplastic bronchial epithelium. The photomicrographs included in Wandall's publication are not very convincing. For example, the epithelioid cells in Fig. 23 of his article appear much more like degenerated ciliated cells; even the cilia are still clearly visible. Those he presented in Fig. 21 resemble young alveolar elements rather than epithelioid cells, while the structures in Fig. 19 look like metaplastic epithelial cells from a bronchus. The dense formation of nuclei which Wandall illustrates in Fig. 24 has the same aspect as the metaplastic giant cells described above.

According to the experience of Stuyt (1947), which corresponds to ours, the cytologic diagnosis of tuberculosis is more easily made in a lymph gland punctate. A picture of epithelioid cells together with a certain amount of necrosis is, in our opinion, characteristic of tuberculosis. Epithelioid cells stain light blue to gray. Their spindle-, or bean-shaped nuclei have a typically delicate and loosely reticulated chromatin and may contain several basophilic nucleoli. It was but once that we encountered unmistakable epithelioid cells in a sputum preparation. The patient had an advanced cavernous tuberculosis of the lung, and numerous acid-fast rods were seen. The rare appearance of these cells in the sputum might be explained by certain differences in the character of pulmonary and lymph gland tuberculosis. In the latter process, proliferation plays a more dominant role, so that tuberculous granulation tissue, and therefore also epithelioid cells, occur in much larger quantities. In the lung the tendency is more in the direction of necrosis and ulceration, especially in those cases where a communication with a bronchial lumen has been established. Besides, the aspect of these cells is rather similar to that of histiocytes and nonphagocytic alveolar cells, and they may easily be mistaken for these nonspecific elements. We thus might have missed the diagnosis in several instances.

Giant cells of Langhans are a rare occurrence even in lymph gland punctates. Whether these cells disintegrate during the process of smearing, or whether they never were anything more than conglomerations of epithelioid cells around a caseated mass is uncertain. Their identification in material derived from the lung or bronchi is very difficult because they so closely resemble the alveolar giant cells. We regard the presence of necrotic material in the center of the structure as a prerequisite for labeling any cell a Langhans' giant cell. In one case of tuberculous bronchitis we saw a giant cell in a biopsy contact smear which met this requirement (Fig. 9). The nuclei, numbering about 30, were round, oval, or shoe-shaped, and presented the typical loosely-reticulated chromatin network of epithelioid cells. The center of the cytoplasm displayed the necrotic ground substance already described above: a polychromatic mass containing numerous red, azurophilic granules.

BRONCHUS ADENOMA

It was only twice that we had the opportunity of studying histologically verified cases of adenoma of the bronchus. Contact smears were prepared from biopsy material of both patients, and the morphology of the two series was identical. The sputum did not reveal any atypical cells, which again supports the idea that nonmalignant epithelial tissue does not exfoliate.

The cells of the bronchus adenoma were cuboidal or pear-shaped (Fig. 10), and clear differences in the extent of differentiation appeared in our smears. The nuclear-cytoplasmic ratio was adequate. Sharply delineated, oval nuclei were situated eccentrically toward one pole of the cell. The chromatin was similar to that of a lymphocyte nucleus, in that it was compact and rarely exhibited granules. One or two basophilic or chromophobe nucleoli were sometimes seen in the nucleus. The cytoplasm, which was sharply demarcated in the well preserved cells, stained lightly and was gray in color. Secretory vacuoles were sometimes noted.

Here and there, some large cells with hyperchromatic nuclei and abnormally shaped, basophilic nucleoli could be seen. However, the general uniformity of the majority of the cells and the absence of other signs of malignancy seemed to justify the diagnosis of benign tumor in both our cases.

CHAPTER V

A CYTOLOGIC CLASSIFICATION OF PRIMARY BRONCHUS AND LUNG CARCINOMA

Bronchogenic and lung carcinomas are well known to have a very polymorphic histologic structure. Even a single section may present two entirely dissimilar pictures in different locations. Likewise two biopsies taken from the same tumor at different times may bear no resemblance to each other. For that reason many workers feel that the classification of a certain bronchus tumor, examined from a given biopsy at a given time, is valueless and of no significance for the prognosis of the disease.

We have nevertheless attempted to correlate the cell morphology of the different tumors with their histologic structure, with the aim of formulating a suitable cytologic classification of lung tumors. We believe that such a classification, if it is possible, would be of great value in the cell diagnosis of cancer. Evidently, the recognition of a particular carcinoma in the structure of cells or cell groups can by itself be an aid in establishing malignancy. The oat cell carcinoma admirably illustrates the point.

The structural polymorphism referred to above is well reflected in cytologic material. Thus it happened on several occasions that a slide showed a cell picture which was regarded typical for more than one tumor. When the cytologic study was repeated at a later date, completely different cells could be found. Even samples of varying origin (e.g., sputum, endobronchial material) taken from the same patient were sometimes so unlike as to make all comparison impossible.

In addition, cells of one tumor type also display considerable pleomorphism. Lastly we had to take into consideration the changes which a tumor undergoes on its way to anaplasia.

Anaplasia, histologically speaking, signifies loss of the ability to differentiate. This refers not only to cell arrangement, such as loss of tubule formation in the adenocarcinoma, but also to changes which

the cytologist can observe; for example, inability to keratinize, loss of clear cellular boundaries, and finally total absence of cytoplasm. In the case of anaplastic carcinoma a specific diagnosis is not always easy, but by searching for some differentiated cells the origin of the tumor may be discovered.

In examining strongly pleomorphic cell pictures we attempt to gain an over-all impression and to recognize at the same time specific characteristics known from more uniform patterns. Compare the distorted cell in Fig. 24 with those of Fig. 15. The chromatin structure of the nuclei is very similar in the two cases. In Fig. 24 one can even detect the beginning of keratinization, as indicated by the peculiar, granular substance at the periphery of the cytoplasm. The cells of Fig. 15 are typical for cornifying squamous cell carcinoma, and that of Fig. 24 was shown to have the same origin.

The question of recognizing cells from a metastatic tumor is one which we cannot treat adequately as our experience with such cases is too limited. We can report, however, that in three cases of metastases in the lung (a thyroid carcinoma, an osteosarcoma, and a chorionepithelioma), the cytologic preparations revealed cells without any resemblance to those of primary bronchus or lung neoplasms (Fig. 59). The cytology of adenocarcinoma of the stomach is likewise known to differ markedly from that of a bronchogenic adenocarcinoma.

With the expansion of our knowledge in this field it might become possible to differentiate primary from secondary pulmonary neoplasms. If the origin of the latter is then to be determined, an excellent working knowledge of the malignant cytology of other organs becomes imperative.

We will proceed with a description of the various malignant cell types as seen in May-Grünwald-Giemsa preparations. The following more or less histologic classification will serve as a basis for the discussion.

CARCINOMAS ARISING FROM THE BRONCHIAL EPITHELIUM

Well differentiated:
(a) keratinizing squamous cell carcinoma
(b) adenocarcinoma
Incompletely differentiated:
(c) squamous cell solid carcinoma
(d) cylindrical cell solid carcinoma

(e) small cell solid carcinoma
Anaplastic:
(f) small cell anaplastic carcinoma
(g) large cell anaplastic carcinoma

CARCINOMAS ARISING FROM THE ALVEOLI

(h) alveolar cell carcinoma
(i) adenocarcinoma arising from the alveoli.

The tumors included under (e) and (f) are known as oat cell carcinoma. Our classification of alveolar tumors is somewhat hypothetical, being based on only two patients with carcinomas originating in the alveoli.

KERATINIZING SQUAMOUS CELL CARCINOMA
(Figs. 11–38)

The outstanding histologic feature of this tumor is the formation of epithelial pearls. Cytologically it is marked by considerable polymorphism. Cells in different stages of development are regularly encountered.

The younger cells are round, with a large nucleus and a narrow margin of cytoplasm. The older ones are squamous cells with very uneven outlines. Now and then, large, sometimes cornified, spindle-shaped cells are seen, which have been called "cigar cells" in Anglo-American literature (Farber et al., 1950).

The nucleus occupies a central position and often has an irregular and bumpy circumference. In most cases the nuclei are round and, typically, flattened or indented on one side. Very characteristic is the coarsely reticulated chromatin structure. The older nuclei display pyknotic changes. A few basophilic nucleoli of medium size may be seen in the younger cells, but there is otherwise no nucleolar pathology.

The well delineated cytoplasm stains intensely basophilic in the young stages, turning light blue and finally light gray with further differentiation. The process of keratinization begins here in young, immature cells, in strong contrast to ordinary cornifying squamous epithelium where it is restricted to mature cells. In view of the fact that the steps in the keratinization of cancer tissue somewhat parallel those in the epidermis, we regard it useful to apply the same terminology. The cells of the stratum granulosum of the skin contain a granulated substance in their cytoplasm called keratohyalin. In the stratum lucidum the lightly staining eleidin is found, while the fibrous stratum corneum contains the actual keratin.

The first evidence of cornification in the young cells of a squamous cell carcinoma is the appearance of a violet, granulated substance which forms a band at the periphery of the cell and may extend through the entire width of the cytoplasm (Fig. 13, 15). In preparations stained with Congo red and hematoxylin this band displayed the same color as keratohyalin (Fig. 16). With Papanicolaou's method it became grayish blue but did not manifest its granular character. We regard these *prekeratin bands* as an almost absolute indication of a keratinizing squamous cell carcinoma, especially when they occur in immature cells.

In the more differentiated cells the granulated substance disappears, making way for a uniform or fibrous, sky-blue material, *keratin*. The latter has a bright orange color in Papanicolaou slides and is strongly eosinophile in hematoxylin-eosin smears.

Vacuolation of the cytoplasm, as a degenerative phenomenon, is seen mainly in cornified cells. Phagocytosis of polymorphonuclear leukocytes occurs with special frequency in this tumor. Autophagocytosis in the lung is practically restricted to the squamous cell carcinoma. It may result in the formation of bird's-eye cells, structures discussed in a previous chapter as possibly related to epithelial pearl formation. Uni- or multinucleated giant cells occur now and then. Necrosis is a frequent complication which sometimes prevents a reliable diagnosis.

ADENOCARCINOMA
(Figs. 39–47).

Histologic examination of this tumor reveals a tubular structure which may or may not be papillomatous. In our cytologic preparations we never saw a definite acinar arrangement although there was a tendency to form irregular cell islands.

The cell shape is mostly columnar, cuboidal, or polygonal, and the edges are rounded off. The somewhat eccentric nucleus is usually longer than wide. Its outline is often irregular, lobulation appearing at times. Pronounced hyperchromatism is frequently seen. In contrast to the squamous cell carcinoma, it is attended with a more compact chromatin structure in which coarse fragments may sometimes be distinguished. The well differentiated cells contain rather few nucleoli. However, with the beginning of dedifferentiation, multiple basophilic nucleoli, which may assume huge dimensions, become evident.

Almost all the cells have a large amount of basophilic cytoplasm, which is more poorly demarcated than in the fully differentiated squamous cell carcinoma. Numerous vacuoles may be found in the

cytoplasm. They sometimes merge to form a large vesicle, pushing the nucleus to the periphery. These vacuoles, in the absence of signs pointing to degeneration, may be regarded as secretory, considering that adenocarcinomas in the lung often produce mucin.

In seven cases of adenocarcinoma, in which contact smears from biopsies and lung punctates were made, we attempted to demonstrate the mucin by applying the reaction of Hotchkiss (1948). This reaction depends on the oxidation of polysaccharides with periodic acid, whereby the carbon bond between the 1—2 glycol groups is broken and a high molecular polyaldehyde is formed. The aldehydes are then made evident with Schiff's reagent, with the result that structures containing mucin are stained red. Realizing that various carcinomas produce a water-solluble mucin, we adopted the modification of using an alcoholic solution of periodic acid.

The reaction of Hotchkiss is actually not specific for mucin. However, as a result of the usual fixation with methyl alcohol or the alcohol-ether combination, only those of the substances giving positive Hotchkiss reactions remain which are high-molecular. They are lipids, polysaccharides (viz., glycogen, mucopolysaccharides and mucoproteins), and prekeratin.

The lipids dissolve during treatment with alcohol and leave the cell. Prekeratin, judging from control smears of a keratinizing squamous cell carcinoma, appears as a red, homogeneous mass in the cytoplasm. An ordinary Giemsa preparation also makes it possible to exclude prekeratin. Glycogen can be identified as red, angular fragments or splinters. By avoiding any contact of our adenocarcinoma slides with alcohol solutions less than 70 per cent, we found it possible to demonstrate the mucus in the form of red, smoothly bounded droplets in an unstained cytoplasm (Figs. 44, 45).

Five of the seven cases showed mucus in the cells, one result was dubious, and one negative. We also had the opportunity of applying this method to sections from a surgical specimen of one of these patients. Numerous red droplets were seen in the cells and in the lumina of the tubules (Fig. 46).

The alcoholic periodic solution has the following composition:

periodic acid	*0.4 gm.*
distilled water	*10 ml.*
0.2 N sodium acetate . . .	*5 ml.*
absolute alcohol	*35 ml.*

The solution becomes inactive after a few days.

SQUAMOUS CELL SOLID CARCINOMA

Histologically this tumor is a nonkeratinizing squamous cell carcinoma, the cells being grouped in nests or cords. Examining the material cytologically one meets, almost exclusively, the young cells described under the heading of the keratinizing carcinoma. They are often arranged here in compact groups. Well differentiated cells with large amounts of light gray cytoplasm and pyknotic nuclei are found only occasionally. The latter resemble the normal superficial squamous epithelial cells rather closely and may offer difficulty in identification. Nevertheless, the increased nuclear-cytoplasmic ratio and the great polymorphism of nuclei, betray the malignant origin of these cells.

CYLINDRICAL CELL SOLID CARCINOMA
(Fig. 48).

In sections this tumor exhibits cuboidal or polyhedral cells which seldom if ever form tubules, but are arranged mostly in nests. In smears the cells are very much like those of the adenocarcinoma, except that they are smaller and less polymorphic. The cytoplasm is less abundant and contains no secretory vacuoles. The nuclei are often situated eccentrically.

In our opinion the small cell, or oat cell carcinomas may be grouped under two headings: a more differentiated solid carcinoma, and an undifferentiated, or anaplastic type. Although preparations often display combinations of the two, they are sufficiently different in their typical forms to warrant the distinction.

SMALL CELL SOLID CARCINOMA
(Figs. 50, 51).

This differentiated oat cell carcinoma consists of small cells displaying great variation in size. The average cell is one to three times as large as a lymphocyte. The nuclei are for the most part irregularly round, and the nuclear-cytoplasmic ratio is very large. The chromatin stains with notable differences of intensity in the several cells and has a typically fragmented structure. Large basophilic nucleoli are sometimes seen, and are surrounded by chromatin condensations.

The cytoplasm, which stains intensely blue, is situated around the nucleus as a thin, fairly well demarcated band. Mitoses are a frequent picture in this tumor. The cells bear a strong resemblance to those of a lymphosarcoma. Only once did we note autophagocytosis.

The pathology report on a biopsy from which the contact smear

presented a picture of only this differentiated cell type was: atypical oat cell carcinoma.

SMALL CELL ANAPLASTIC CARCINOMA
(Figs. 52–54).

Small, dark, round, or spindle-shaped cells mark the histologic picture of this tumor. In the smear, bare nuclei of the same average size as those of the differentiated oat cell carcinoma are scattered throughout. Pathognomic for this cell type is the extremely irregular outline of the nuclear border. There may be indentations, protruding lobules, and even segmentation of the nuclear matter. The cause of this phenomenon may probably be found in the degeneration of the nuclear membrane, whereby the nucleus collapses in the smear, producing a rather artificial change. If the material has been handled with too much force, nothing may remain beyond some lightly staining, ragged structures.

The staining intensity of the chromatin, here too, is variable. It often presents a streaky aspect or is condensed into fragments. The sparse nucleoli are rarely basophilic but mostly chromophobe. Necrosis is very common.

LARGE CELL ANAPLASTIC CARCINOMA
(Figs. 55–57).

This cancer, which we have named so to distinguish it from the small cell anaplastic carcinoma, has a mixed origin. On several occasions we examined cytologic smears which showed cells of the large cell anaplastic carcinoma in combination with those of a squamous cell carcinoma, and once with those of an adenocarcinoma. Also the histologic sections of the latter tumor revealed the structure of an anaplastic carcinoma in which only sporadic tubules were seen.

The cells from the completely anaplastic parts consist entirely of free nuclei, whose size may vary. Most of these are oval and their borders are much more even than in the oat cell carcinoma. The chromatin is characterized by a delicate granular reticulum. Very typical are the polymorphic, irregular, poorly defined nucleoli which stain basophilic and are sometimes immensely large. Giant nuclei are a constant finding in this tumor. The nuclei are frequently embedded in a necrotic ground substance.

NEOPLASMS ARISING FROM THE ALVEOLI

A rare form of primary lung neoplasm is pulmonary adenomatosis, a tumor which has been demonstrated by serial sections to originate in the alveoli. Its morphology is strikingly similar to abnormalities

found in the lung in the course of the so-called "jagziekte". This disease, which occurs in sheep, is probably caused by a virus.

Adenomatoses are benign tumors but may become malignant (Bos; 1951). This being the case, one essentially deals with an alveolar cell carcinoma, although there is no agreement among the various workers as to whether all alveolar cell carcinomas may be grouped under one heading. The histologic structure of the benign and malignant form is that of an adenoma and adenocarcinoma, respectively. In both conditions flame-shaped tumor cells line the alveolar septa.

Two cases of lung tumors originating in the alveoli are at our disposal. The first had the histologic structure of an adenomatosis. Dr. P. Lopes Cardozo examined the sputum of this patient and found malignant cells which he considered derived from an alveolar cell carcinoma because of their resemblance to normal alveolar cells. The histologic diagnosis in the second case was "alveolar cell tumor". However, when we examined it cytologically, there was absolutely no similarity between this and the first tumor. We regarded the cells of the second neoplasm as typical for adenocarcinoma of the bronchial epithelium.

These observations illustrate several points. In the first place cytologic examination may differentiate between two neoplasms which emanate from the alveoli and look very much alike in histologic sections. Secondly the findings suggest that our tumors had the following origins: the first arose from the alveolar cells. Considering the histiocytic character of the latter, one could even call it an alveolar cell sarcoma. Bos (1951), however, regards "carcinoma" a suitable term because of the tubule formation.

The second tumor might have been an adenocarcinoma from respiratory epithelium that had migrated into the alveoli (assuming, of course, that there was no coexisting primary bronchus carcinoma in some other place). Such a migration has been described in several chronic processes in the lung. These new growths of alveolar origin thus provide further evidence for what we have previously suggested; namely, that the alveolar wall, under pathologic conditions, may have a cell lining of varying origin (Chapter IV).

ALVEOLAR CELL CARCINOMA
(Fig. 58).

The cell picture of the tumor classified histologically as adenomatosis and cytologically as alveolar cell carcinoma, was extremely polymorphic. There were very large and very small cells, some multinucleated, shaped like a candle flame or a circle sector.

The nuclei were round, oval, or bean-shaped and exhibited the same loose chromatin pattern that characterizes normal alveolar cells. The average cell contained one or two large, basophilic nucleoli. The eccentric location of the nucleus at the border of the cytoplasm gave these cells their typical aspect. Due to this phenomenon one gets the impression, especially under low power magnification, of dealing with alveolar cells.

The cytoplasm, which usually stained polychromatic and sometimes strongly basophilic, was very abundant, and made for a rather normal nuclear-cytoplasmic ratio in spite of the large size of the nuclei. In some of the cells it contained numerous vacuoles, suggesting active production of mucin. In view of the fact that we had only sputum for our analysis, we did not undertake to stain for mucin.

The investigations reported above have made it apparent that clear cytologic distinctions can be made between at least four types of primary bronchus and lung carcinomas; namely, the squamous cell, cylindrical cell, and small cell carcinomas of the bronchus, and an alveolar cell carcinoma.

Moreover, one can observe various stages in the direction of anaplasia, which in its ultimate form may present two entirely different pictures: the small cell anaplastic carcinoma with its single origin, and the large cell anaplastic carcinoma, which may develop from one of several tumors.

CHAPTER VI

AN EVALUATION OF CYTOLOGIC DIAGNOSIS

If one is to get the correct impression of the reliability of the cytologic method of diagnosis, two factors must be taken into account: (1) the reliability of the method itself, that is, the criteria of malignancy, and (2) the certainty of such a diagnosis established on a basis of a given material. Many cytologists seem to make the mistake of evaluating their method by comparing the results of cytologic examination of one type of specimen, e.g., sputum, bronchial secretions, etc., with histologic findings from a completely different type. They consequently report figures which comprise both items without estimating each individually. The material which the pathologist has at his disposal, such as biopsy tissue, a resected lung, or even a cadaver, is much more suitable and more representative than what the cytologist receives for routine examination. Nevertheless, the relative inferiority of the samples does not detract from the value of cytologic analysis.

To orient ourselves concerning the reliability of our criteria, we have made many contact smears of biopsy tissue which was subsequently sent to the pathologist. Cytologist and histologist thus inspected identical material. *The result was that all samples considered malignant, or strongly suspected of being malignant by the histologist were independently diagnosed as such by us.* This does not imply that the diagnoses "malignant" or "suspicious" were always the same in a given case.

We have already expressed our view on the value of a given material, by comparing our results in sputum analyses with those in other types of specimens (see Chapter II).

In the course of 28 months, cytologic examinations were made of 428 patients. Ninety-six of these were proven by histologic analysis to be suffering from bronchus or lung cancer. Fourteen other malignant cases were established with reasonable certainty by clinical methods (bronchography, tomography, bronchoscopy).

In Table IV the results of our cytologic analyses of different material are summarized, and compared with the diagnoses from biopsy tissue obtained by bronchoscopy.

TABLE IV

The cytologic findings in 82 histologically, and 15 clinically established cases of bronchogenic and pulmonary malignancy, compared with the results of histologic examination of biopsy material.

	Diagnoses in 82 histologically proved cases of malignancy			Diagnoses in 14 clinically established cases of malignancy			Diagnoses in a total of 96 cases of malignancy		
	+	±	Total	+	±	Total	+	±	Total
Cytologic report	73	8	81	11	2	13	84	10	94
Histologic report on biopsy	68	3	71	–	–	–	68	3	71

+ = conclusive evidence of malignancy
± = suspected malignancy

It will be noted that of the 332 negative or unproven cases of lung cancer, another 15 were found positive, and one suspicious, by means of cytologic examinations. Their case histories, however, could not be followed, partly because the patients refused to subject themselves to further examination. Among these were also cases sent to us by the outpatient department or by private physicians, so that their records were not at our disposal.

The incidence of the various lung tumors, as we encountered them in cytologic smears, was as follows:

Squamous cell carcinomas and the anaplastic carcinomas derived therefrom	46 cases	(49 %)
Oat cell carcinomas	18 „	(19 %)
Adenocarcinomas and the anaplastic carcinomas derived therefrom	8 „	(8,5 %)
Anaplastic carcinomas which could not be further specified	10 „	(11 %)
Alveolar cell carcinomas	1 „	(1 %)
Metastatic lung tumors	5 „	(5 %)
Unclassified tumors	6 „	(6,5 %)
	94 cases	(100 %)

Forty-nine per cent of the cases thus appeared to belong to the pathologically more favorable group of squamous cell carcinoma.

The correlation between histologic and cytologic diagnoses is illustrated in the following survey of histologically proven cases of bronchogenic or pulmonary malignancy. Instances of epithelial metaplasia are also listed here.

KEY TO ABBREVIATIONS

Ca. = carcinoma
Sq.c.ca. = squamous cell carcinoma
Sq.c.sol.ca. = squamous cell solid carcinoma
Oatc.ca. = oat cell carcinoma
Adenoca. = adenocarcinoma
Cylc.sol.ca. = cylindrical cell solid carcinoma
Anapl.ca. = anaplastic carcinoma
La.c.an.ca. = large cell anaplastic carcinoma
Alv.c.ca. = alveolar cell carcinoma
Met. = metastatic
Sa. = sarcoma
+ = conclusive evidence of malignancy
± = suspected of malignancy

No.	Cytology	Histology
1.	+ adenoca.	+ alv.c. tumor
2.	+ oatc.ca.	± ca.
3.	+ sq.c.ca.	+ sq.c.ca.
4.	+ sq.c.ca.	+ ca.
5.	+ sq.c.ca.	+ sq.c.ca.
7.	+ sq.c.sol.ca.	+ ca.
8.	+ sq.c.ca.	+ sq.c.ca.
10.	+ la.c.an.ca.	+ anapl.ca.
11.	+ anapl.ca.	+ splintering ca.
12.	+ partly anapl., partly sq.c.ca.	+ sq.c.ca.
13.	+ anapl.ca.	+ ca.
14.	+ sq.c.ca.	+ sq.c.ca.
15.	+ sq.c.ca.	+ sq.c.ca.
16.	+ sq.c.ca.	+ sq.c.ca.
17.	+ oatc.ca.	+ oatc.ca.
18.	+ sol.ca.	+ met.anapl.ca.
20.	+ alv.c.ca.	adenomatosis
22.	+ sq.c.ca.	+ atypical sq.c.ca.
23.	± sq.c.ca.	+ sq.c.ca. without keratinization

No.	Cytology	Histology
25.	± ca.	+ sq.c.ca.
26.	+ anapl.ca.	+ anapl.sol.ca.
27.	+ malignancy	+ malignant blastoma
29.	+ sq.c.ca.-sol.ca.-la.c.an.ca.	+ sq.c.ca.
31.	+ malignancy	+ osteogenic sarcoma left knee
32.	+ met.? ca.	± ca.
34.	metaplasia and chronic inflammation	chronic inflammation
38.	+ partly anapl.ca.	+ ca.
40.	metaplasia	inflammation
42.	metaplasia	tuberculous cavity
43.	+ adenoca.-cylc.sol.ca.	+ adenoca.
44.	+ atypical ca.	+ sol.ca.
46.	+ oatc.ca.	+ anapl.ca.
47.	metaplasia	tuberculosis of the lung
48.	+ sq.c.sol.ca.	+ sq.c.ca.
51.	+ met. thyroid ca.	± malignant tumor
52.	+ sq.c.ca.	+ sq.c.ca.
53.	+ sq.c.ca.	+ sol.ca.
57.	+ oatc.ca.	+ oatc.ca.
58.	+ sq.c.ca.	+ sq.c.ca.
59.	+ sq.c.ca.	+ sq.c.ca.
60.	+ adenoca.	+ adenoca.
61.	+ oatc.ca.	+ oatc.ca.
62.	+ sq.c.ca.	+ sq.c.ca.
63.	+ sq.c.ca.-la.c.an.ca.	+ sq.c.ca.
64.	+ sq.c.ca.-anapl.ca.	+ sq.c.ca.
65.	+ sq.c.ca.	+ sq.c.ca.
66.	± ca.	+ oatc.ca.
67.	+ adenoca.-cylc.sol.ca.	+ sol.ca. with sporadic tubules
69.	+ sq.c.ca.	+ sq.c.ca.
70.	+ oatc.ca.	+ oatc.ca.
72.	+ sq.c.ca.	+ ca.
73.	+ sq.c.sol.ca.	+ papillary sq.c.ca.
75.	+ oatc.ca.	+ anapl. bronchogenic ca.
76.	+ sq.c.ca.	+ sq.c.ca.
77.	+ differentiated oatc.ca.	+ oatc.ca.
78.	+ sq.c.ca.-sol.ca.-la.c.an.ca.	+ sq.c.sol.ca.
82.	+ sq.c.ca.? adenoca. ?	+ sq.c.ca.

No.	Cytology	Histology
84.	+ oatc.ca.	+ oatc.ca.
88.	± oac.ca.	+ oatc.ca.
92.	± ca.	+ anapl.ca.
93.	+ la.c.an.ca.	± ca.
94.	metaplasia, necrosis	corpus alienum
95.	± ca.	+ sol.ca.
96.	± ca.	+ bronchogenic ca.
97.	metaplasia	tuberculosis
99.	+ cells suspicious of sa.	+ met. osteogenic sa. in both lungs
101.	+ sq.c.ca.	+ sq.c.ca.
102.	metaplasia	pulmonary fibrosis
103.	+ adenoca.-cylc.sol.ca.	+ anapl.ca.
104.	+ sq.c.ca.-la.c.an.ca.	+ sq.c.ca.
106.	+ sq.c.ca.	+ sq.c.ca.
107.	+ met. chorionepithelioma	+ chorionepithelioma of the testis
108.	+ sq.c.ca.-sq.c.sol.ca.-la.c.an.ca.	+ sq.c.ca.
109.	+ oatc.ca.	+ oatc.ca.
110.	adenoma	adenoma
111.	+ ca., oatc.ca.?	+ oatc.ca.
112.	+ adenoca.-cylc.sol.ca.	+ ca.
113.	polymorphic, ciliated epithelium	granulomatosis
114.	+ la.c.an.ca.	+ anapl.ca.
115.	+ entirely differentiated oatc.ca.	+ atypical oatc.ca.
116.	+ oatc.ca.	+ bronchogenic ca.
118.	+ sq.c.ca.	+ ca.
122.	+ sq.c.ca.	+ sq.c.ca.
124.	+ sq.c.ca.	+ atypical ca.
126.	+ oatc.ca.	+ anapl.ca.
127.	± sq.c.ca.	+ sq.c.ca.
130.	+ sq.c.sol.ca.	+ atypical ca.
131.	+ adenoca.	+ adenoca.
134.	+ ca., sq.c.ca.?	+ sq.c.ca.
135.	+ sq.c.sol.ca.	± sq.c.ca.
136.	adenoma	adenoma

SUMMARY

This work presents the results of cytologic investigations on 428 patients suspected of carcinoma of the bronchus or lung. By means other than cytologic analysis, malignant growth was ascertained in 96 of these.

In the Introduction a survey of the literature on clinical cytology of lung and bronchus cancer is given. Its history is traced back to 1887 when Hampeln first applied this diagnostic procedure.

In Chapter I several possible ways of gaining cytologic material are discussed. The examination of sputum is of considerable practical value, especially in that no instrumental intervention is necessary. It has its disadvantages, however: loss of time, appreciable degeneration of the cells, and a rather high percentage of false negatives. The endobronchial paths may be explored with the aid of a bronchoscope or a Métras tube. By these means, bronchial secretions can be aspirated, a cotton swab may be rubbed against the tumor or bronchial mucosa, or pieces of tissue can be excised and touched directly to a slide (contact smears). Since this material is rich in well-preserved cells, a much lower percentage of false negatives is attained. If the tumor is peripheral and does not communicate with the bronchial tree, there still remains the possibility of a diagnostic transpleural lung puncture.

In Chapter II some of the current techniques of preparation are reviewed and evaluated. Wet fixation, as opposed to dry fixation, is shown to have definite advantages only when used in association with hematoxylin as a nuclear stain. With the aid of cytochemical procedures, the specificity for nucleic acids of the May-Grünwald-Giemsa and Papanicolaou staining methods was determined. Smears of an anaplastic carcinoma were used for that purpose and were subjected to fractionated hydrolysis in 1 N hydrochloric acid. The slides were then treated by the above methods and according to the Feulgen and the methyl green-pyronine techniques. The results of the

experiment indicate that the May-Grünwald-Giemsa method, in contrast to Papanicolaou's, differentiates between the several nucleic acid components of the nucleus and cytoplasm, although neither procedure demonstrates nucleic acids exclusively. Apart from that, the first method has the greater affinity for slightly or unpolymerized nucleic acids. It is consequently better suited for bringing out those cellular structures associated with ribonucleic acid and slightly polymerized desoxyribonucleic acid. Since a detailed cell picture is of utmost importance in revealing cytologic evidence of malignancy, the Giemsa technique seems the method of choice. This procedure is also simple, and facilitates comparison with other branches of clinical cytology, e.g., lymph gland puncture.

In an effort to gain some of the advantages of both Papanicolaou's and Giemsa's method, the hematoxylin-eosin-azure technique of Maximow was modified. Although sharp definition of the chromatin is obtained, the stain is more transparent than panoptic.

Chapter III gives a systematic account of the general and specific characteristics which smears of malignant cells may present. It is emphasized that degenerative and regenerative phenomena on the one hand, and malignant growth on the other must be well differentiated. In view of the fact that routine clinical work is usually concerned with stages of malignant growth far removed from the point of transition between malignant autonomous and reactive growth, certain quantitative changes in cell structure and cell relation may be regarded specific.

In Chapter IV the cells of the oral, pharyngeal, and tracheobronchial epithelium are described. An account is presented of the cell changes seen during degeneration and regeneration of the respiratory epithelium. Replacement by a nonspecific squamous epithelium (metaplasia) is considered a source of false reports of malignancy by those unfamiliar with the aspect of its constituent cells.

The morphology of the alveolar cells is then described, and the problem of their origin discussed. Their structure, along with their property of migrating intact into the lumen of the alveoli and phagocytose particles, speaks for a mesenchymal origin. The cells constituting the epithelial lining of the alveoli under pathologic conditions may be derived from several sources. Among these are respiratory or metaplastic epithelial cells from the bronchi which have migrated into the alveoli, or hyperplastic, and possibly metaplastic alveolar cells.

The cytology of two nonmalignant diseases of the respiratory tract, pulmonary tuberculosis and bronchial adenoma, is next considered. In the former condition it was found difficult to demonstrate epithelioid cells in the sputum and to differentiate Langhans' giant cells from alveolar or metaplastic giant cells.

In Chapter V an attempt is made to correlate the histologic structure of bronchus and lung carcinomas with their cell morphology. Four different tumor types can be differentiated on a basis of cytology: squamous cell carcinoma, cylindrical cell carcinoma, small cell carcinoma, and alveolar cell carcinoma. Moreover, several grades of anaplasia can be distinguished. Two neoplasms were examined in which the pathologic report indicated an alveolar origin. Cytologically, however, one was an alveolar cell carcinoma and the other a bronchogenic adenocarcinoma. To explain this discrepancy it is assumed that the first tumor arose from alveolar cells, while the second came from respiratory epithelium which had migrated into the alveoli.

In Chapter VI the cytologic method of cancer diagnosis is discussed. It is stressed that the reliability of the criteria of malignancy and the various sources of the specimens must be evaluated independently of each other. Comparison of results from a given material with those from an inferior material was therefore avoided.

Cytologic examination of the same specimens that were subsequently sent to the pathologist made it evident that a diagnosis of malignancy can be safely made on a basis of the cytologic criteria. Of 96 cases of bronchus and lung carcinoma, 94 were detected cytologically by means of samples from any of the sources cited above. No false positive reports were made in this series.

The distribution of the malignant cases among the various types of tumors is shown, after which all the cytologic diagnoses are listed and compared with those of the pathologist.

REFERENCES

Albertini, A. von. (1945), Schweiz. Z. f. Pathol. und Bact.: *8*, 289.
Zur Anwendung der Phasenkontrast Mikroskopie in der Pathologischen Histologie.

Albertini, A. von. (1947), ibid.: *10*, 4.
Vergleichende histologischen Geschwulstuntersuchungen mit dem Phasenkontrastverfahren.

Appel, M. and *Bronk, T. T.* (1949), Am. J. Clin. Pathol.: *19*, 323.
Tumor cells in bronchial secretions.

Bessis, M. (1948), Cytologie sanguine normale et pathologique, p. 47. Paris: Masson & Cie.

Bloom, W. Personal communication.

Bos, S. E. (1951), Thesis Amsterdam.
Over alveolaircel tumoren.

Caspersson, T. O. (1950), Cell growth and cell function: p. 101–151. New York: Norton & Co. inc.

Clerf, L. H. and *Herbut, P. A.* (1950), Ann. Rev. Tuberculosis: *61*, 60.
The value of cytological diagnosis of pulmonary malignancy.

Deelman, H. T. (1952), Ned. Tijdschr. v. Geneesk.: *96*, 1850.
Beschouwingen over het longcarcinoom.

Doglioni, L. (1951), Rivista di Anatomia Patologica e di Oncologia: *4*, 513.
L'Esame citologico a fresco dell' espettorato col microscopio a contrasto di fase.

McDonald, J. R. and *Woolner, L. B.* (1947), Proc. of the staff meetings of The Mayo Clinic: 22, 3.
Cytological diagnosis of bronchogenic carcinoma.

Dudgeon, L. S. and *Wrigley, C. H.* (1935), The J. of Lar. and Otol.: *50*, 752.
On the demonstration of particles of malignant growth in the sputum by means of the wet-film method.

Dijkstra, B. K. S. (1951), Ned. Tijdschr. v. Geneesk.: *95*, 3627.
De diagnostiek van bronchuscarcinoom.

Farber, S. M. et al. (1948), Des. of the chest: *14*, 663.
Cytologic studies of sputum and bronchial secretions in primary carcinoma of the lung.

Farber, S. M. et al. (1950), Cytologic diagnosis of lung cancer, p. 43. Springfield, Illinois: Charles C. Thomas, Publisher.

Hampeln, P. (1887), St. Petersburger med. Wchnschr.: *17*, 137.
Uber ein Fall von primairen Lungen-Pleura carcinom.

Hampeln, P. (1897), Zeitschr. f. klin. Med.: *32*, 247.
Ueber den Auswurf beim Lungencarcinom.

Herbut, P. A. and *Clerf, L. H.* (1946), The Med. Clin. of N. America: *30*, 1384.
Cancer cells in bronchial secretions.

Hers, J. F. P. and *Mulder, J.* (1951), J. Path. and Bact.: *63*, 329.
Rapid tentative post-mortem diagnosis of influenza with the aid of cytological smears of the tracheal epithelium.

Hers, J. F. Ph. (1951), Ned. Tijdschr. v. Geneesk.: *95*, 1495.
Een geval van fibrosis pulmonum e causa ignota.

Hotchkiss, R. D. (1948), Arch. of Bioch. *16*, 131.
A microchemical reaction resulting in the staining of polysaccharide structures in fixed tissue preparations.

Josselin de Jong, R. de. (1928), Ned. Tijdschr. v. Geneesk.: 72, 5019.
Sputumonderzoek bij boosaardige longgezwellen.

Josselin de Jong, R. de. (1929), ibid.: *73*, 3636.
Histologisch onderzoek als hulpmiddel bij de klinische diagnose der boosaardige longgezwellen.

Keuning, F. J. (1950), ibid.: *94*, 1777.
De cytologische diagnose van long- en baarmoederkanker.

Keuning, F. J. (1951), ibid.: *95*, 2803.
Celdiagnostiek van longkanker.

Koller, P. C. (1948), Nature: *151*, 244.
Origin of malignant tumor cells.

Korson, R. (1951), J. Exp.: Med.: *93*, 121.
A microspectrophotometric study of red cell nuclei during pycnosis.

Korteweg, R. (1953), Ned. Tijdschr. v. Geneesk.: *97*, 806.
Het nut, maar tevens de slechts beperkte waarde van vroege herkenning van kanker in het algemeen en van longkanker in het bijzonder.

Leuchtenberger, C. (1950), Chromosoma: *3*, 449.
A cytochemical study of pycnotic nuclear degeneration.

Lignac, G. O. E. (1939), Ned. Tijdschr. v. Geneesk.: *83*, 4906.
„. . . Mors taciturna docet."

Lopez Cardozo, P. (1950a), ibid.: *94*, 1635.
Het herkennen van gezwelcellen in volgens May-Grünwald-Giemsa gekleurde uitstrijk praeparaten.

Lopes Cardozo, P. (1950 b), ibid.: 94, 2758.
De clinische waarde van het cytologische onderzoek als diagnostische methode.

Lopes Cardozo, P. en *Janssen, C. A. L.* (1950), ibid.: *94*, 2318.
De clinische cytologie van sputum en bronchus curettement.

Mulder, J. en *Hers, J. F. Ph.* (1954), ibid.: *98*, 347.
De mogelijke correlatie tussen foetide bronchitis en „metaplastisch" epitheel in bronchiëctasieën.

Nolting, G. H. (1951), Thesis Leiden.
De anatomie van de bronchiaalboom vooral met het oog op diagnostische mogelijkheden in de peripherie van de long.

Papanicolaou, G. N. and *Cromwell, H. A.* (1949), Des. of the chest: *15*, 412.
Diagnosis of cancer of the lung by the cytologic method.

Pollister, A. W. and *Leuchtenberger, C.* (1949), Proc. Nat. Acad. Sc.: *35*, 111.
The nature of the specificity of methyl green for chromatin.

Richardson, H. L. et al. (1949), Am. J. Clin. Pathol.: *19*, 323.
A cyto histologic study of bronchial secretions.

Saphir, O. (1949), ibid. 19, 310.
Cytologic diagnosis of cancer from pleural and peritoneal fluids.

Stowell, R. E. (1942), J. Nat. Cancer Inst.: *3*, 111.
Photometric histochemical determination of DNA in experimental carcinogenesis.

Stowell, R. E. (1945), Cancer Research: *5*, 283.
Thymo-nucleic acids in tumors.

Stowell, R. E. and *Cooper, Z. K.* (1945), ibid.: *5*, 295.
The relative thymonucleic acid content of human normal epidermis, hyperplastic epidermis, and epidermoid cancer.

Stowell, R. E. (1945 a), Stain Technol.: *20*, 45.
Feulgen reaction for thymonucleic acids.

Stuyt, L. B. J. (1947), Thesis Amsterdam.
De betekenis van de lymphklier-punctie voor de diagnostiek van periphere lymphklierzwellingen.

Swierenga, J. (1949), Ned. Tijdschr. v. Geneesk.: *93*, 3037.
Punctie-biopsie bij longgezwellen.

Swierenga, J. (1951), ibid.: *95*, 2878.
Diagnostische moeilijkheden bij het vermoeden van een bronchusgezwel.

Vaandrager, F. G. (1932), Thesis Utrecht.
Het primair boosaardig longgezwel in Nederland.

Vincent Memorial Hospital, The staff of the. (1950), The cytologic diagnosis of cancer, p. 209–211.
Philadelphia, London: Saunders Co.
Wandall, H. H. (1944), Thesis Kopenhagen.
A study on neoplastic cells in sputum as a contribution to the diagnosis of primary lung cancer.
Woolner, L. B. and *McDonald, J. R.* (1950), Des. of the chest: *17*, 1.
Cytologic diagnosis of bronchogenic carcinoma.
Zinser, H. K. (1949), Zentralbl. Gynäk.: 71, 945.
Zytologische Karzinom diagnose mit dem phasenkontrast verfahren.
Zinser, H. K. (1950), Ztschr. Geburtsh. u. Gynäk.: 133, 74.
Die Vitalzytologische Karzinom-diagnose.

PHOTOMICROGRAPHS

Contents

Fig. 1–10 . . . . . nonmalignant cytology
„ 11–38 . . . . . squamous cell carcinoma
„ 39–47 . . . . . adenocarcinoma
„ 48 . . . . . cylindrical cell solid carcinoma
„ 49–54 . . . . . oat cell carcinoma
„ 55–57 . . . . . large cell anaplastic carcinoma
„ 58 . . . . . alveolar cell carcinoma
„ 59 . . . . . metastatic chorionepithelioma

KEY TO ABBREVIATIONS

Sp. = sputum
Cs. = cotton swab of bronchial mucosa
Cb. = contact smear of biopsy
Co. = contact smear of a surgical specimen
Lp. = lung punctate
Se. = parrafin section of a surgical specimen
M.G. = May-Grünwald-Giemsa technique
Pap. = Papanicolan technique
H.E. = hematoxylin-eosin technique
H.E.A. = hematoxylin-eosin-azure technique

The photomicrographs were taken on Gevaert microgran 35 mm. film.

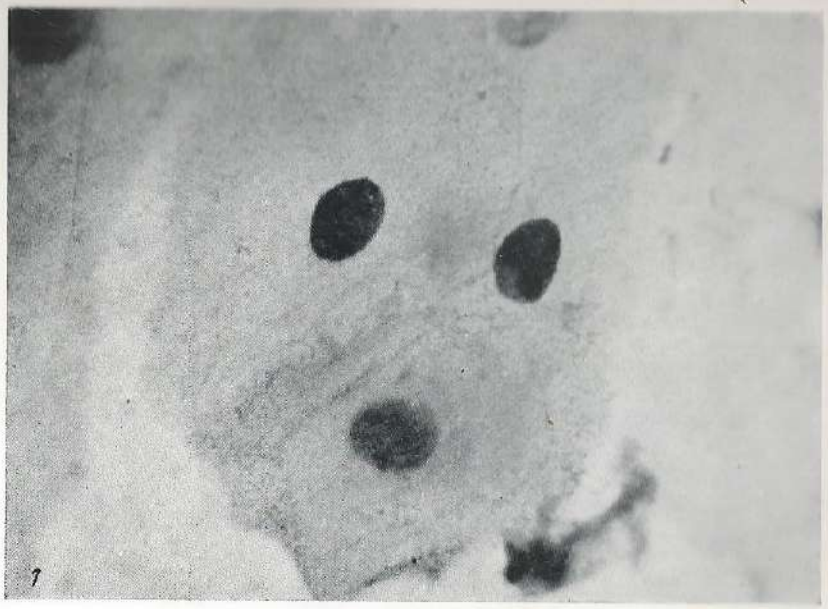
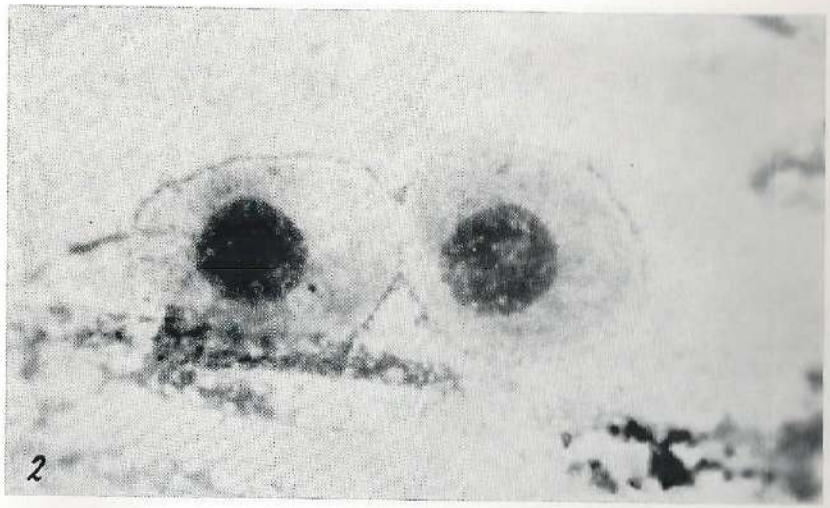


Fig. 1. Superficial cells from a stratified, squamous epithelium.
Sp., M.G. 1000 ×



Sp., M.G. 1350 ×
Fig. 2. Basal cells from a stratified squamous epithelium.

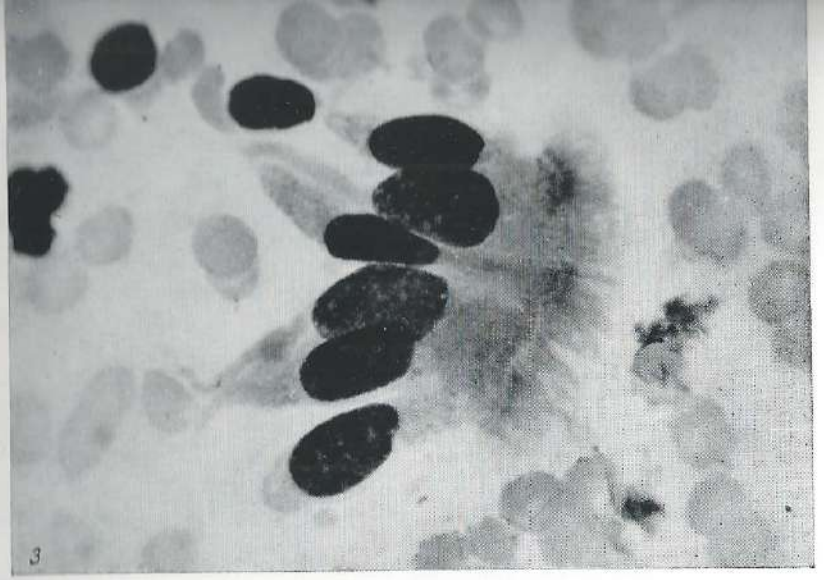


Fig. 3. Ciliated columnar cells from the epithelium of a major bronchus.
Cb., M.G. 1000 ×

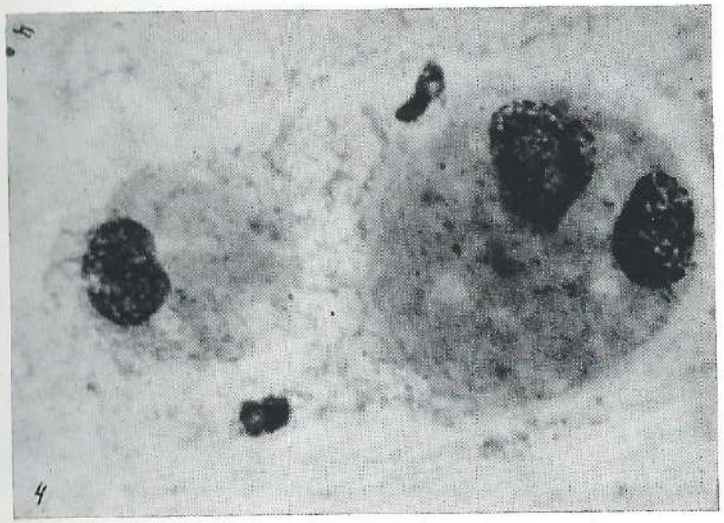


Fig. 4. Alveolar cells (carbon macrophages). Note the double nucleated cell.
Sp., M.G. 1200 ×

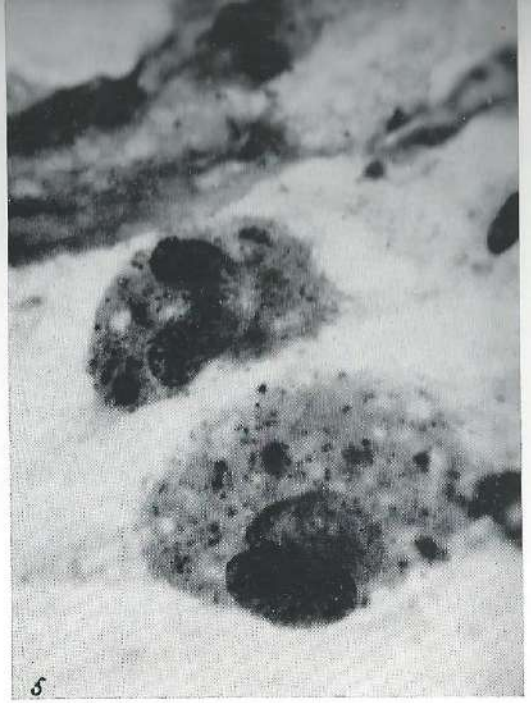


Fig. 5. Alveolar cells with ingested hemosiderin particles (siderophages), from a case of mitral stenosis. The pigment is distributed much more coarsely than the carbon particles in fig. 4.
Sp., M.G. 1200 ×

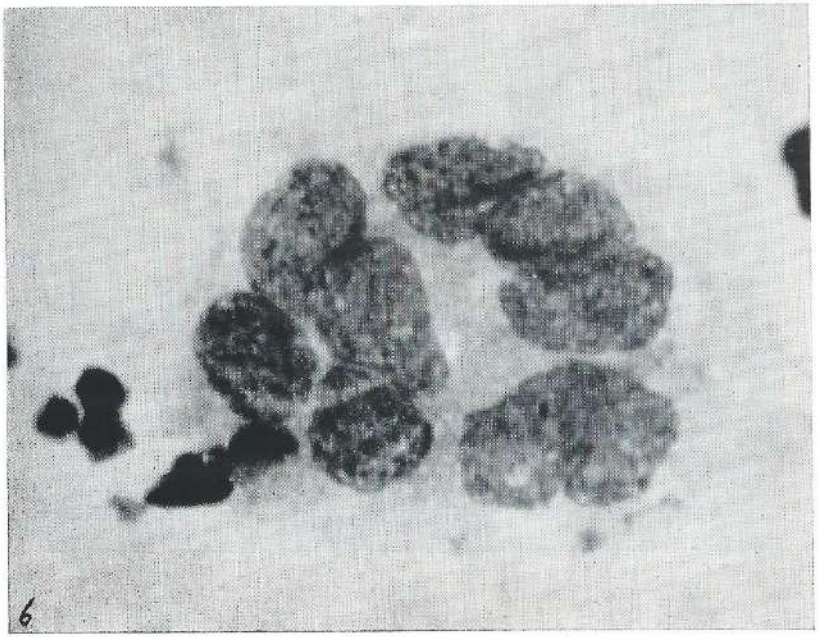


Fig. 6. Alveolar giant cell.
Sp., M.G. 1400 ×

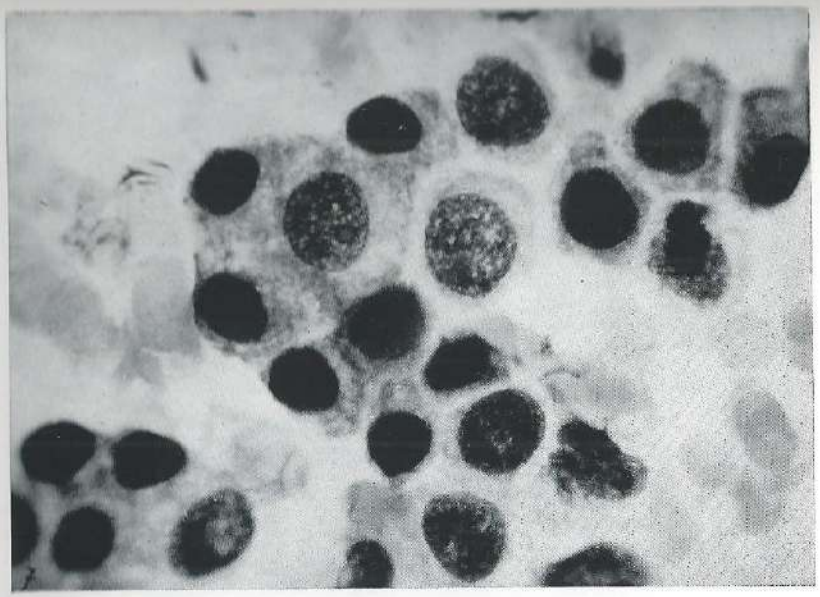


Fig. 7. Cells derived from metaplastic bronchus epithelium. A case of tuberculous bronchitis.
Cb., M.G. 1000 ×

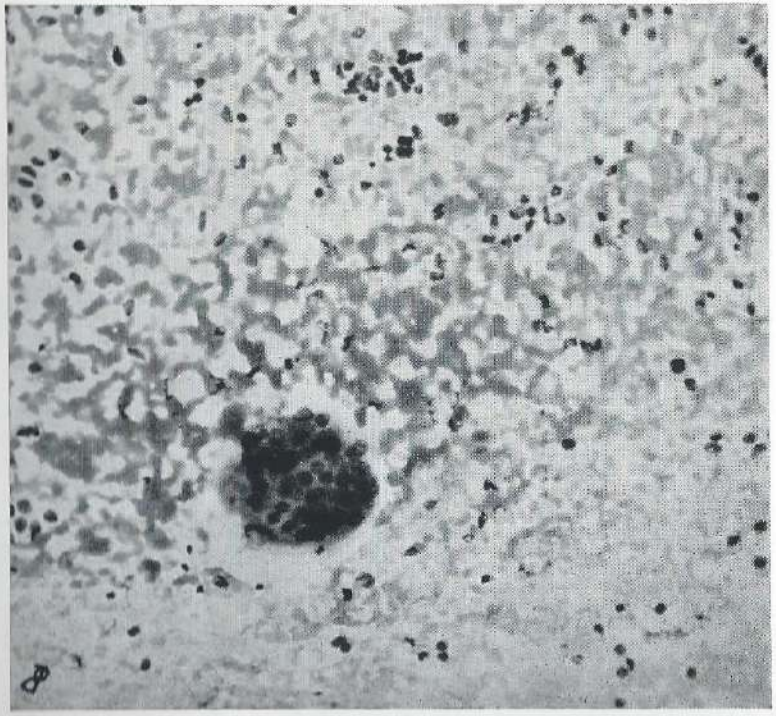


Fig. 8. Metaplastic giant cell.
Cb., M.G. 160 ×

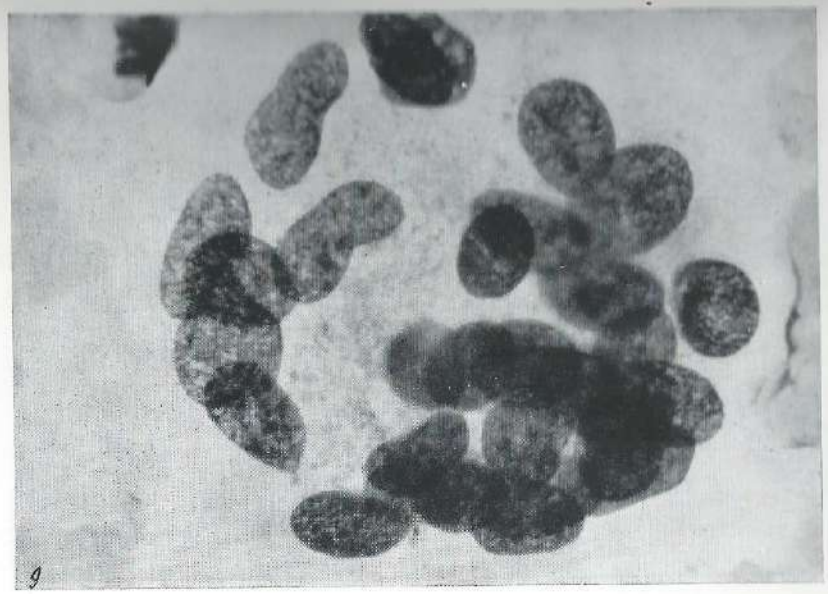


Fig. 9. A possible giant cell of Langhans, from a case of tuberculous bronchitis. The center of the cell consists of a necrotic mass.
Cb., M.G. 1000 ×

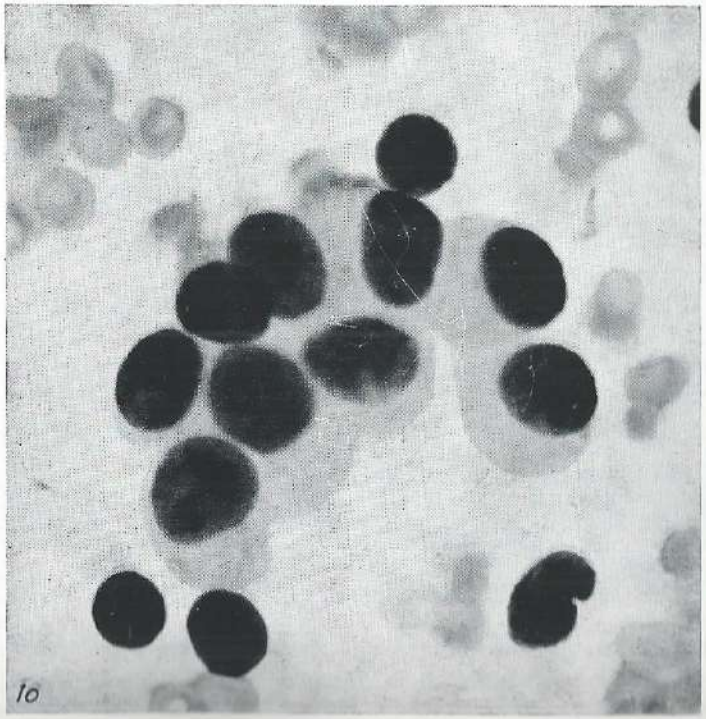


Fig. 10. Cells from a bronchial adenoma.
Cb., M.G. 1200 ×

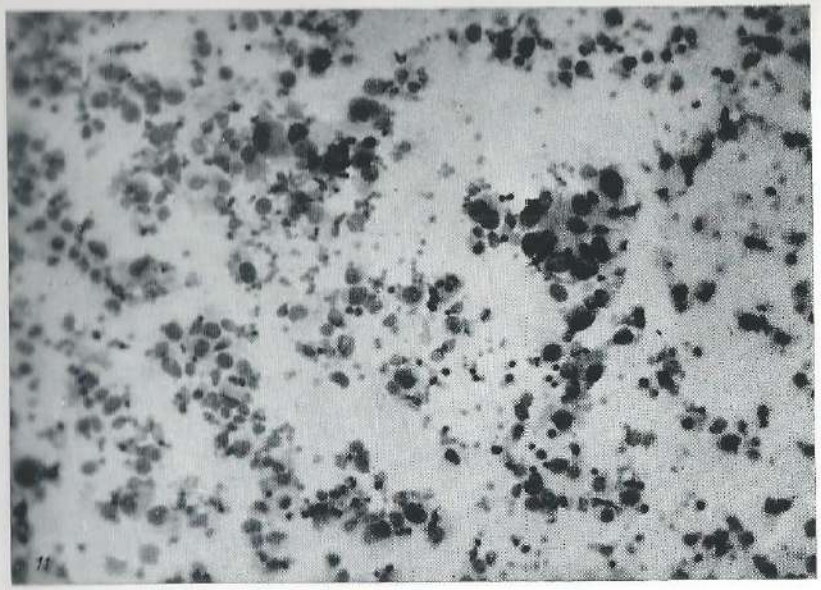


Fig. 11. Squamous cell carcinoma. Note the pronounced polymorphism.
Cs., M.G. 100 ×

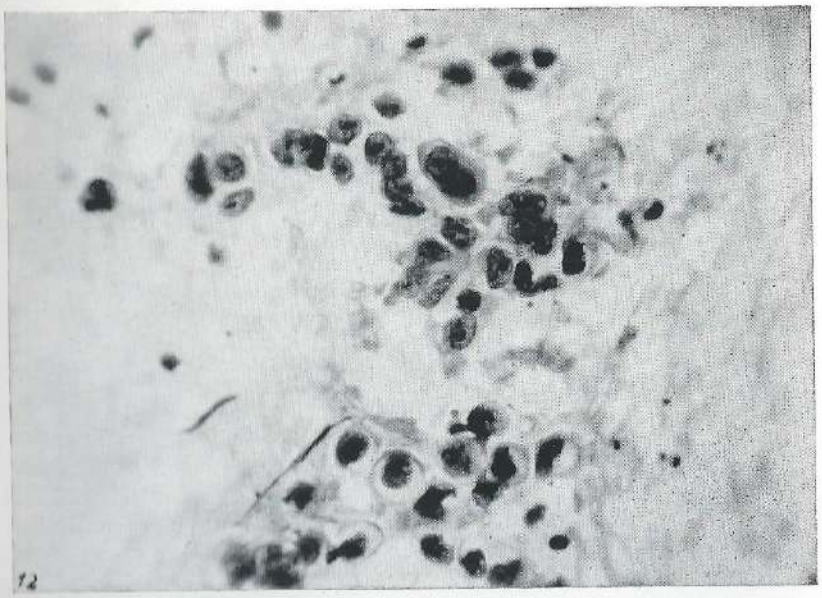


Fig. 12. Squamous cell carcinoma.
Sp., Pap. 400 ×

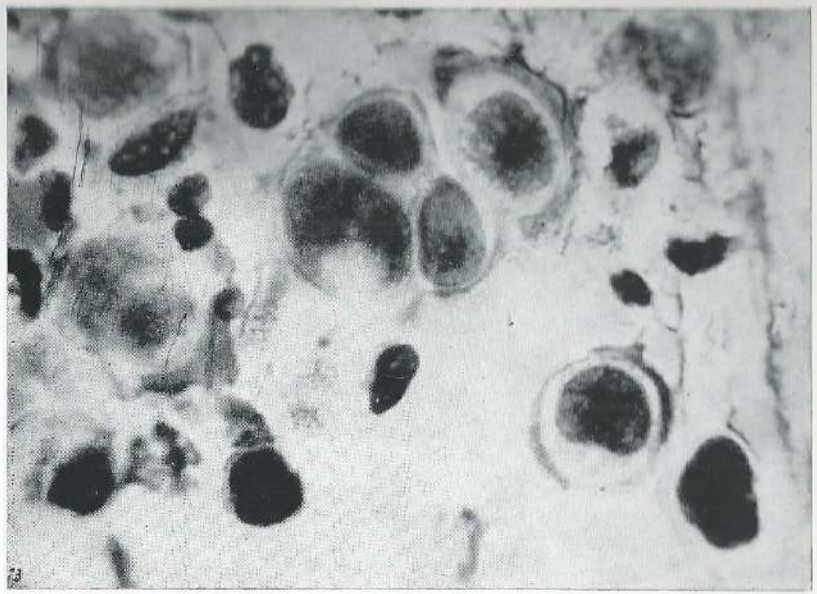


Fig. 13. Squamous cell carcinoma. Note flattened and indented nuclei and prekeratin rings.
Sp., M.G. 1000 ×

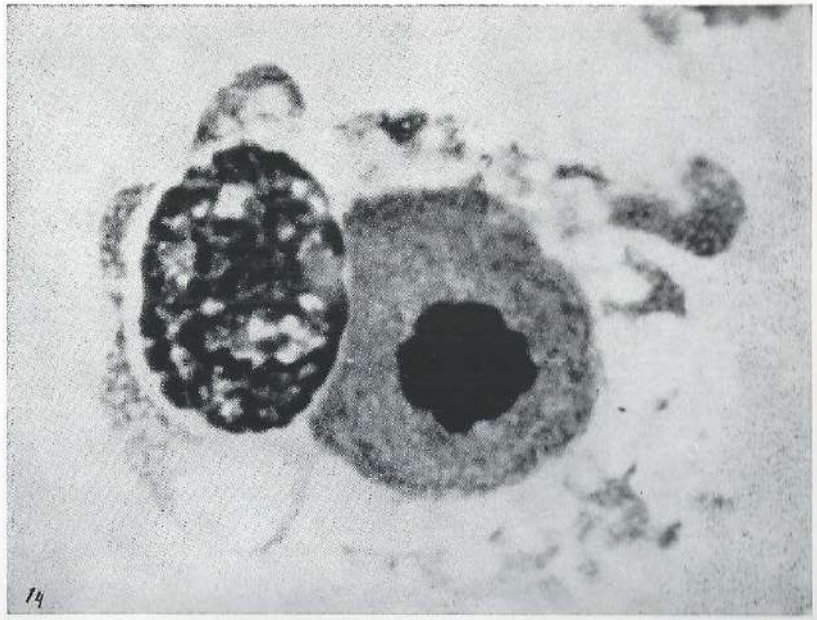


Fig. 14. Squamous cell carcinoma, showing hyperchromatism (left) and pyknosis (right).
Cs., M.G. 1500 ×

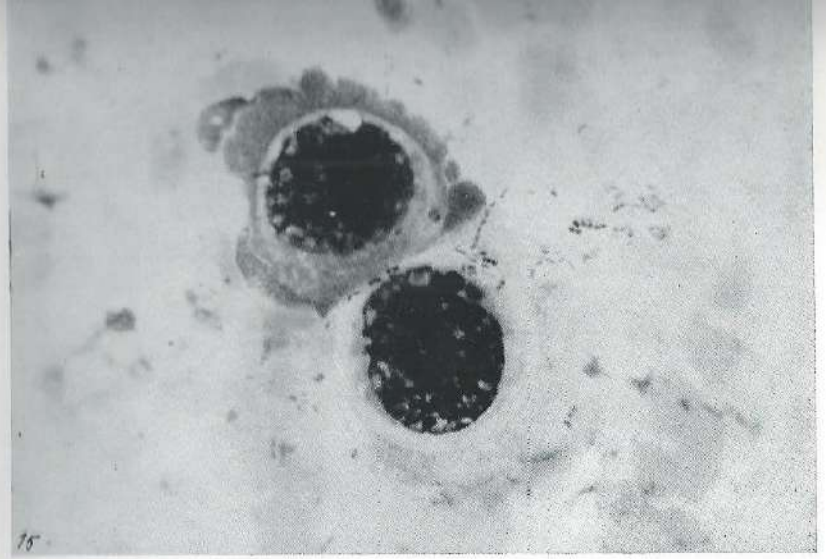


Fig. 15. Squamous cell carcinoma, with granular prekeratin bands and coarsely reticulated chromatin structure of the nucleus.
Sp., M.G. 1000 ×

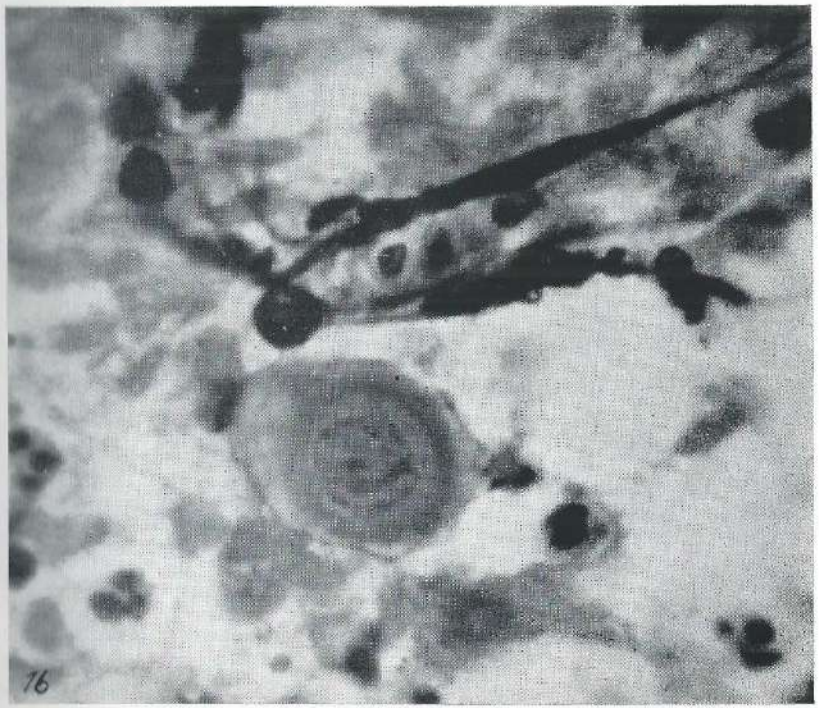


Fig. 16. Squamous cell carcinoma; prekeratin band.
Sp., congo red-haematox.

Fig. 16 60 ×
read: 600 ×

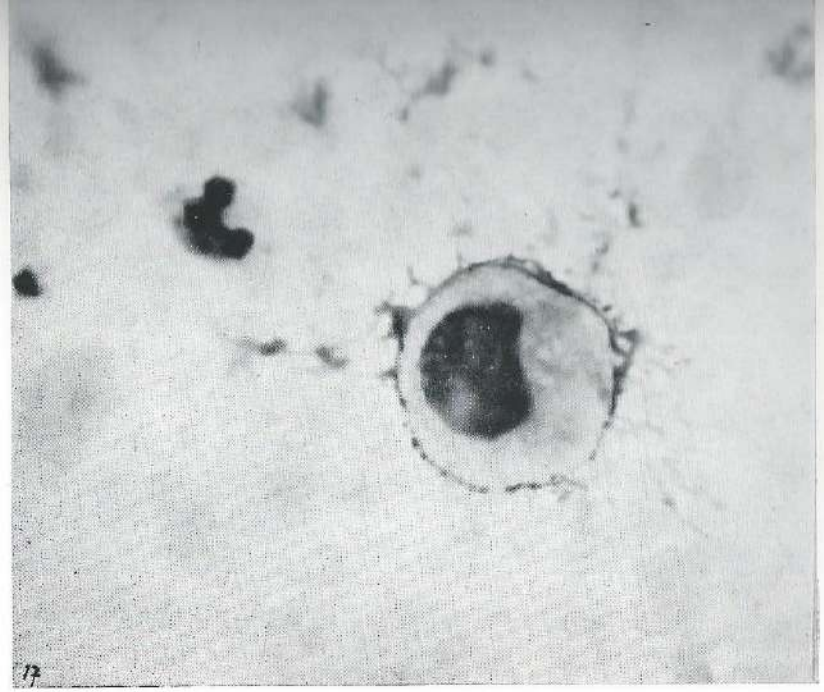


Fig. 17. Squamous cell carcinoma. Note the uniformly keratinized cytoplasm and the indented, pyknotic nucleus.
Sp., M.G. 1200 ×

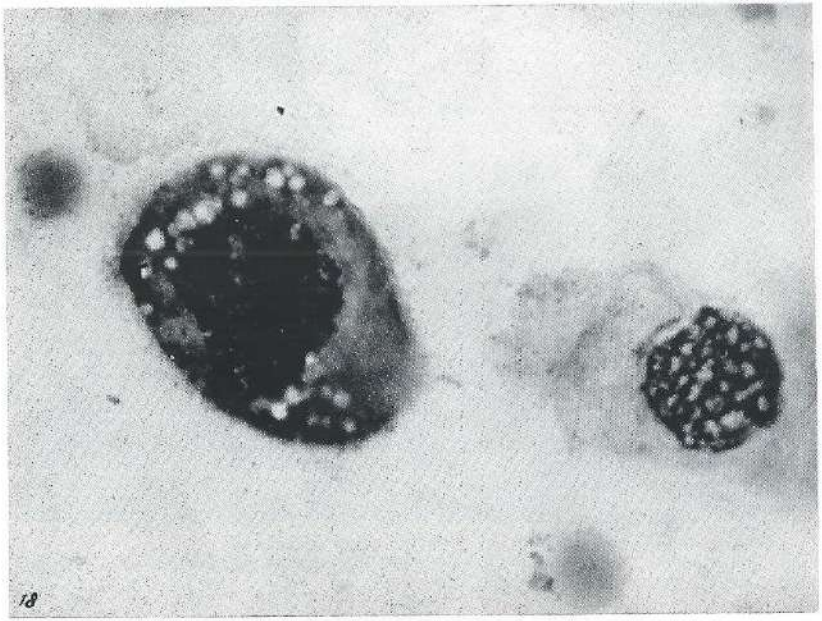


Fig. 18. Squamous cell carcinoma. The cornified cytoplasm exhibits vacuoles due to degeneration. The nuclear structure is typical.
Cs., M.G. 1200 ×

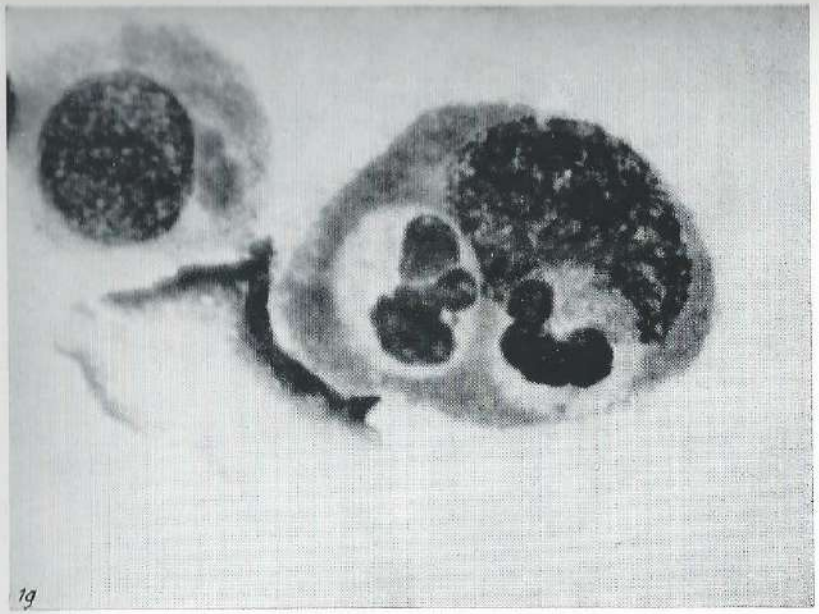


Fig. 19. Squamous cell carcinoma, showing phagocytosed polymorphonuclear leukocytes.
Cs., M.G. 1500 ×

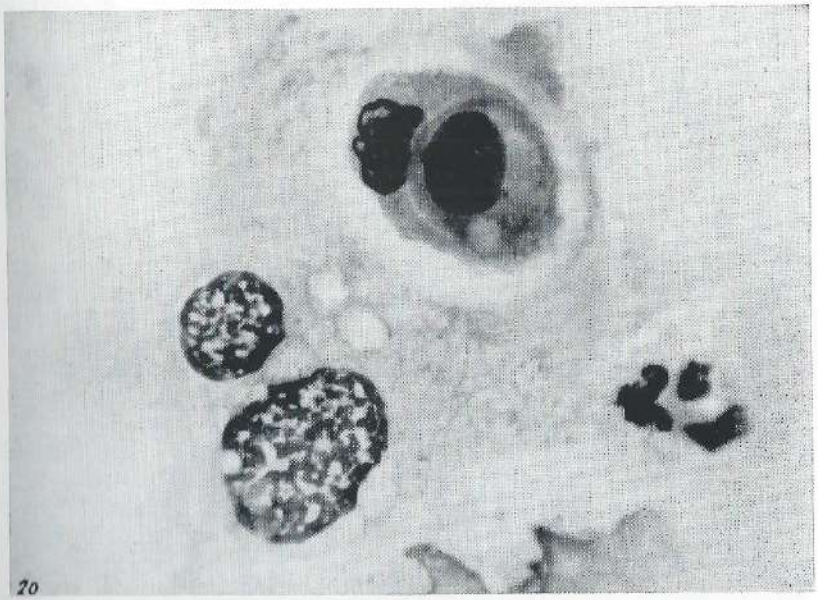


Fig. 20. Squamous cell carcinoma, showing autophagocytosis. A large, double nucleated cell has phagocytosed another phagocytic cell.
Cs., M.G. 900 ×

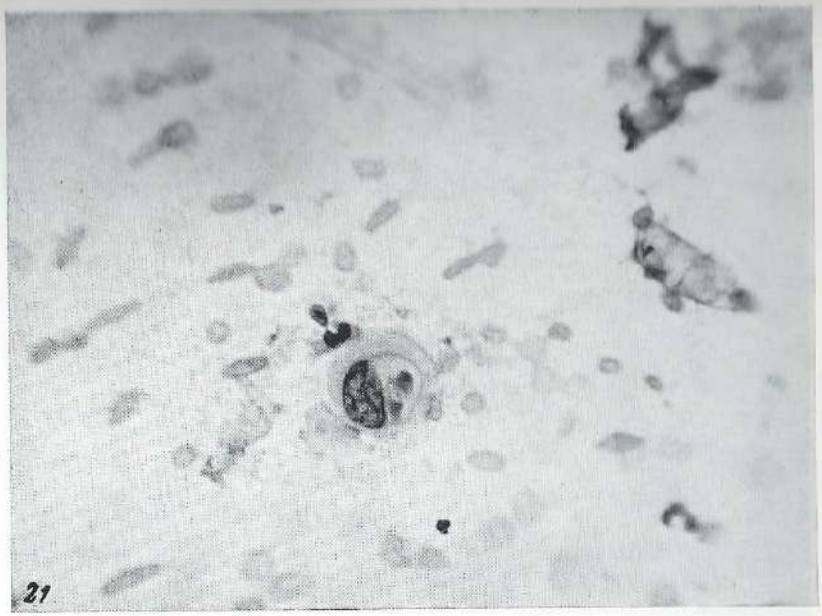


Fig. 21. Squamous cell carcinoma, showing autophagocytosis.
Sp., Pap. 400 ×

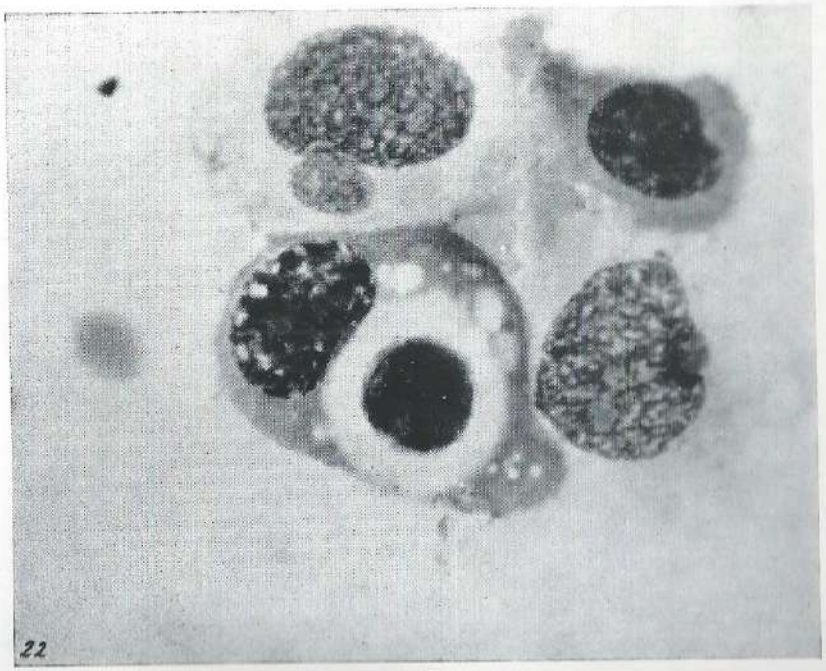


Fig. 22. Squamous cell carcinoma, showing autophagocytosis. The phagocytosed cell has been for the most part digested, producing a typical bird's-eye cell.
Cs., M.G. 900 ×

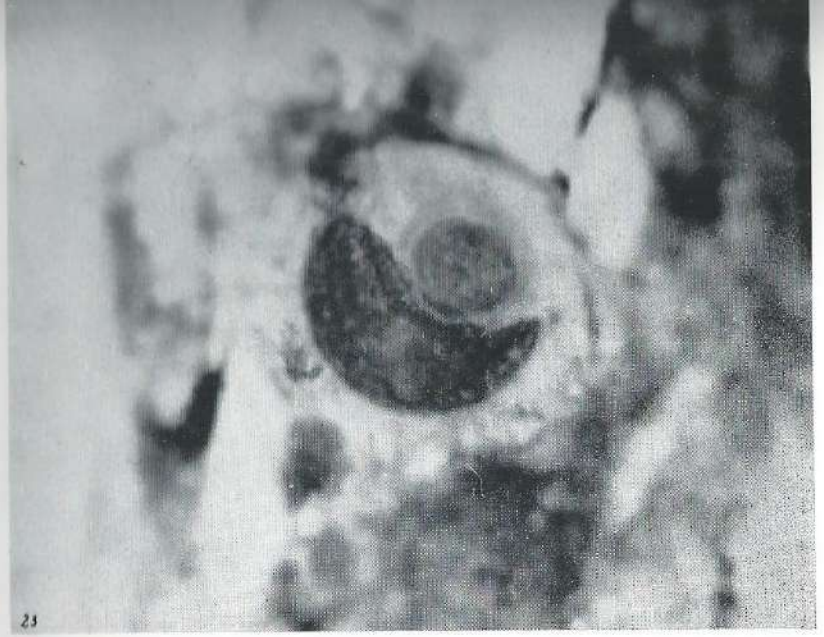


Fig. 23. Squamous cell carcinoma; a bird's-eye cell.
Sp., M.G. 1000 ×

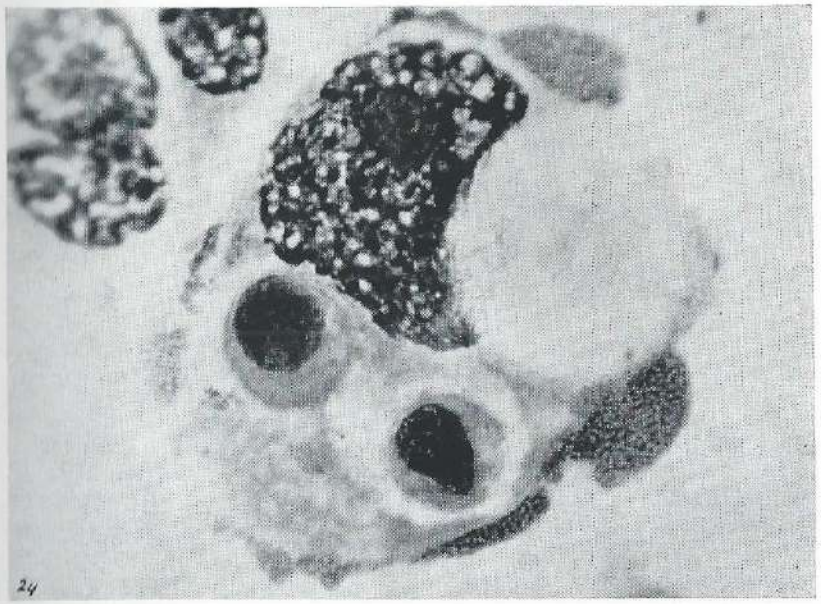


Fig. 24. Squamous cell carcinoma. A uninuclear giant cell has phagocytosed two other malignant cells. A third vacuole is empty. The typical chromatin structure of the nucleus and a prekeratin border at the margin of the cell, betray its origin as a keratinizing squamous cell carcinoma. The nucleus has a large nucleolus which stained blue in the smear.
Cs., M.G. 900 ×

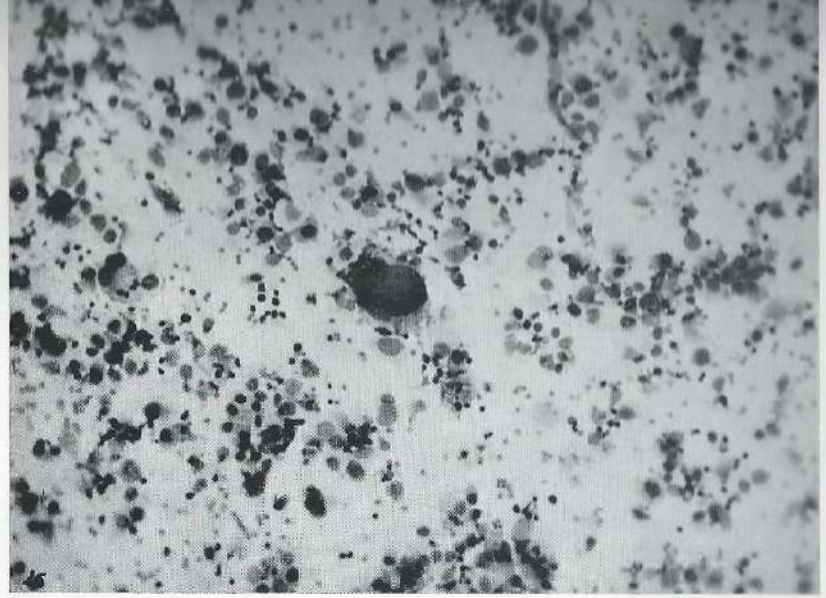


Fig. 25. Squamous cell carcinoma. Note the uninuclear giant cell in the center of the field.
Cs., M.G. 100 ×

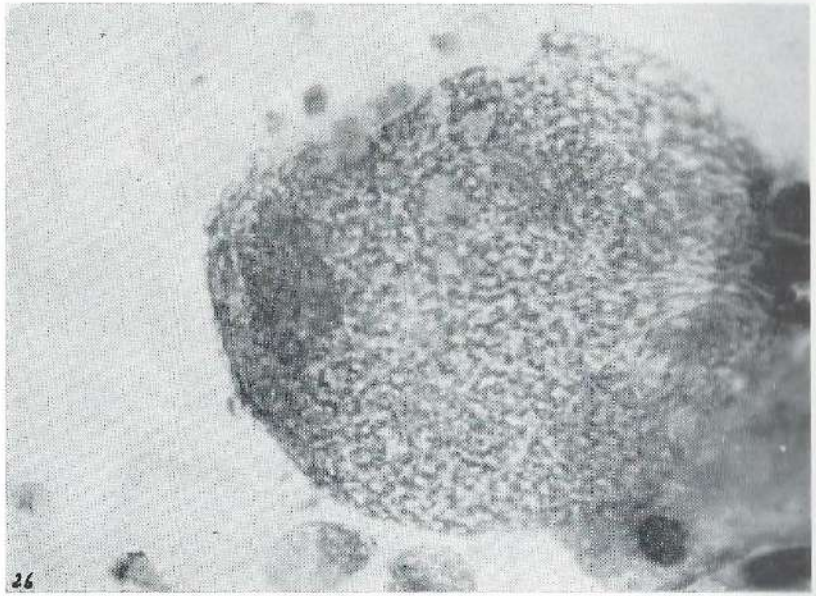


Fig. 26. The giant cell of Fig. 25.
Cs., M.G. 900 ×

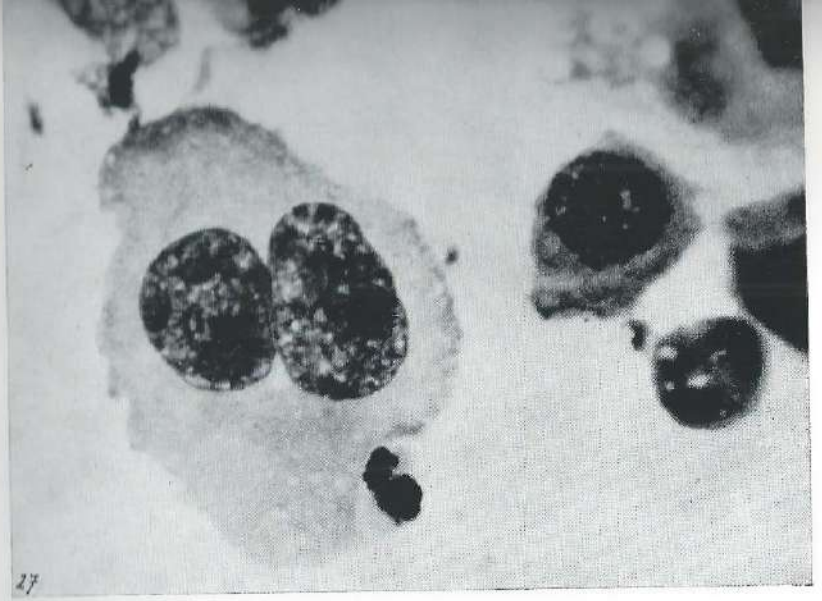


Fig. 27. Squamous cell carcinoma, showing a double nucleated cell.
Cs., M.G. 1200 ×

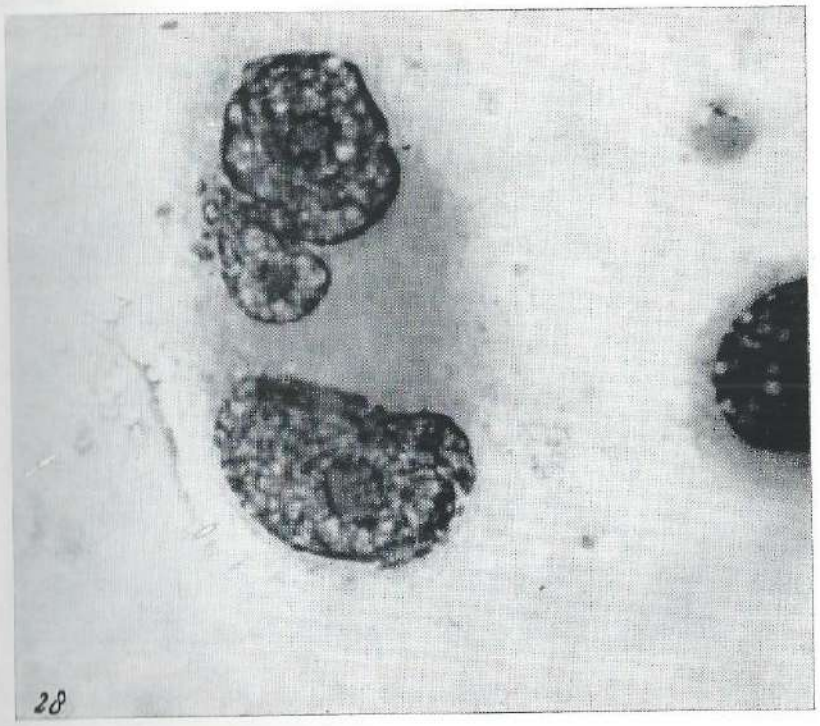


Fig. 28. Squamous cell carcinoma. The giant cell contains three nuclei which are, typically, unequal in size. The poorly defined cell boundary indicates early anaplasia.
Cs., M.G. 1000 ×

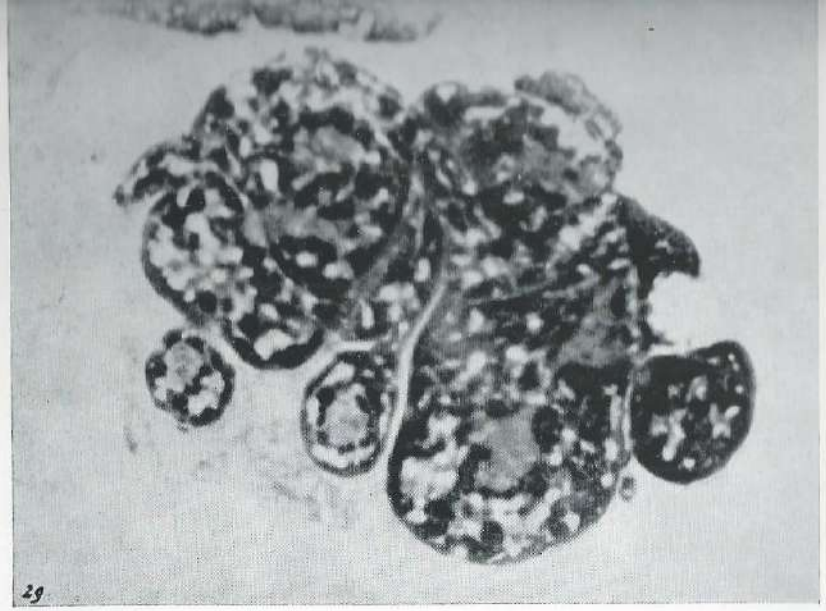


Fig. 29. Squamous cell carcinoma; a multi-nucleated giant cell.
Cs., M.G. 1500 ×

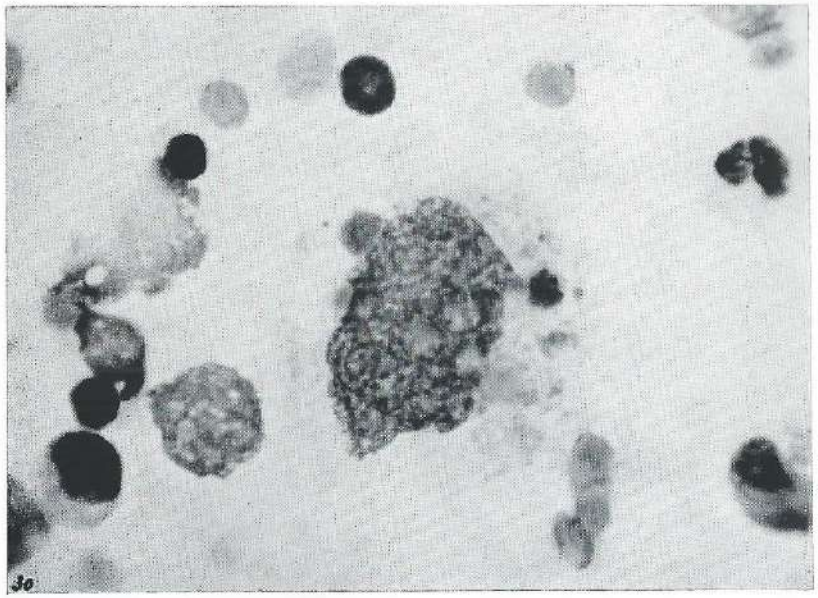


Fig. 30. Squamous cell carcinoma. Note irregular nuclear border.
Cs., M.G. 900 ×

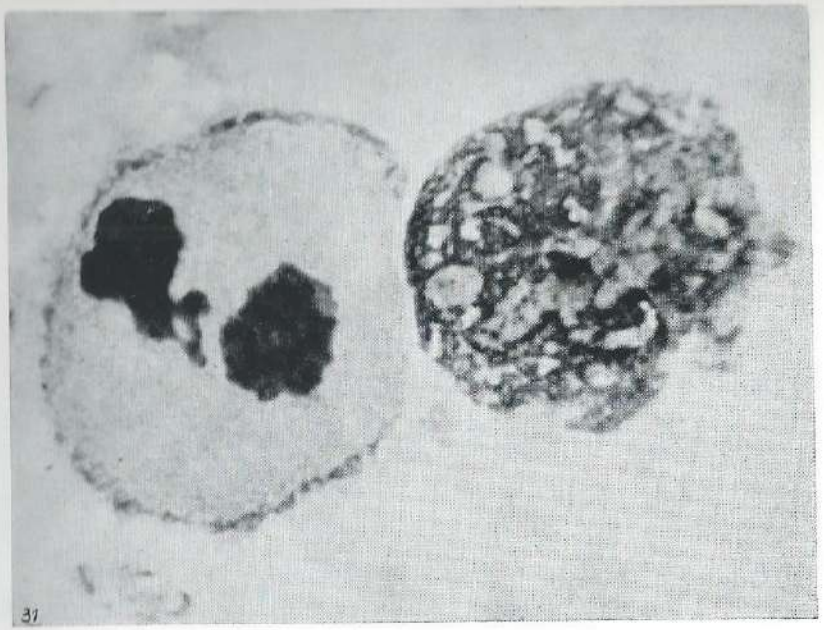


Fig. 31. Squamous cell carcinoma; karyorrhexis (left cell).
Cs., M.G. 1500 ×

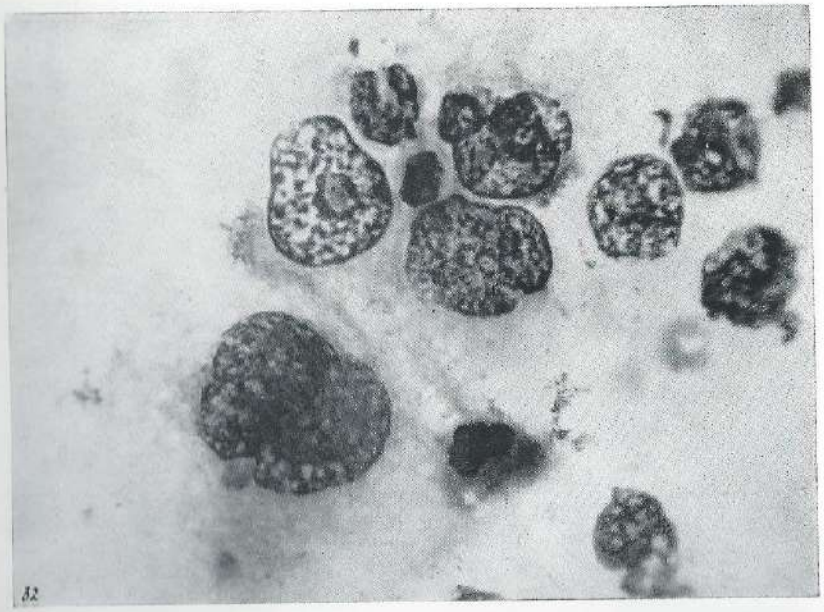


Fig. 32. Squamous cell carcinoma; hyperchromatism of the nuclear border and frame.
Cs., M.G. 900 ×

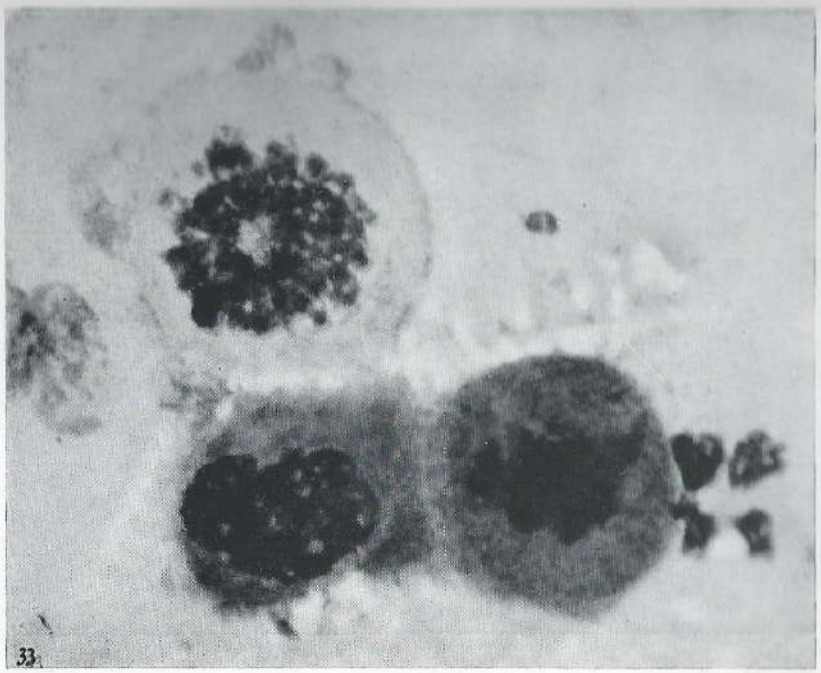


Fig. 33. Squamous cell carcinoma; atypical mitoses.
Cs., M.G. 1200 ×

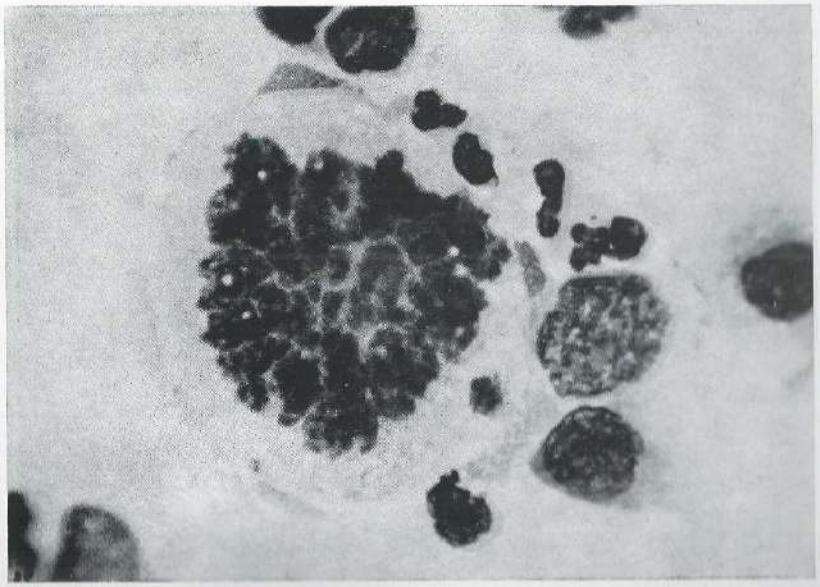


Fig. 34. Squamous cell carcinoma. The giant cell exhibits an atypical mitosis, a large irregular monaster, and clumping of chromosomes. There is evidence of a prekeratin border.
Cs., M.G. 900 ×

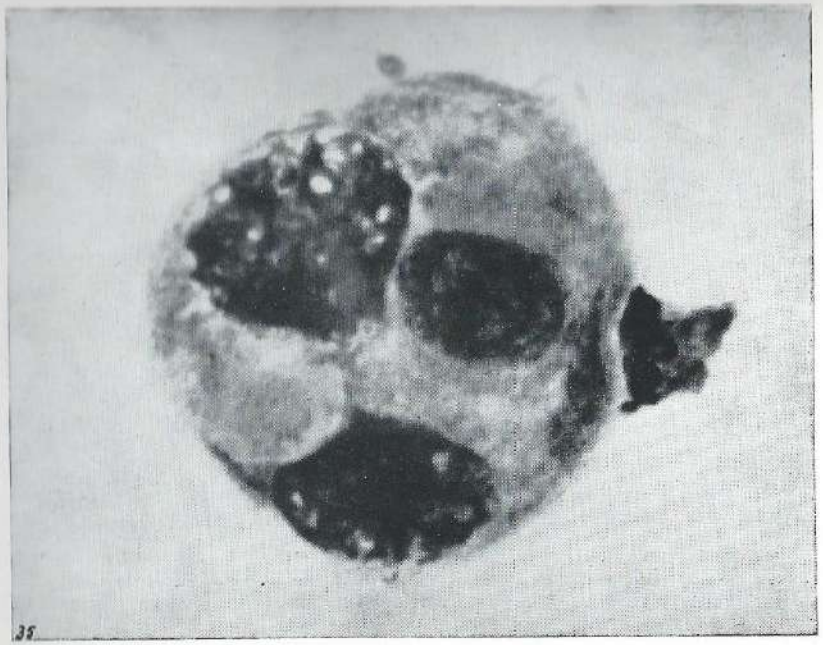


Fig. 35. Squamous cell carcinoma. A tripolar mitosis is seen in the telophase, two nuclei still being connected by a filament of chromatin. The daughter nuclei are unequal in size.
Cs., M.G. 1500 ×

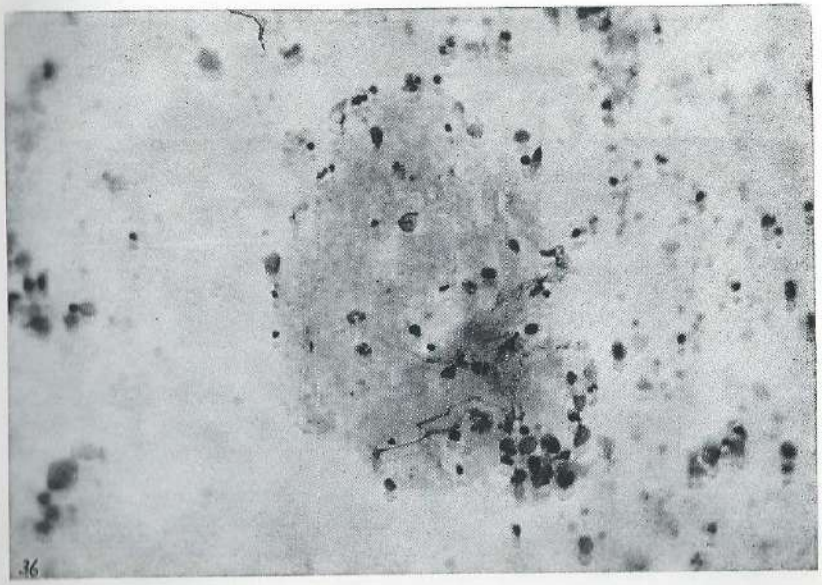


Fig. 36. Squamous cell carcinoma. A patch of necrotic ground substance containing nuclear debris and fine granulations, which stained acidophilic.
Cs., M.G. 150 ×

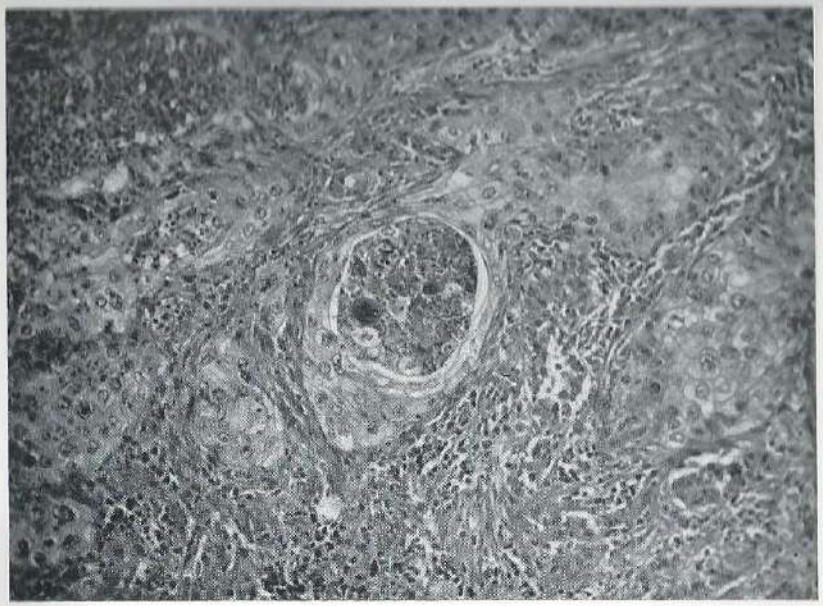


Fig. 37. Squamous cell carcinoma, showing epithelial pearl with a softened center.
Se., H.E. 100 ×

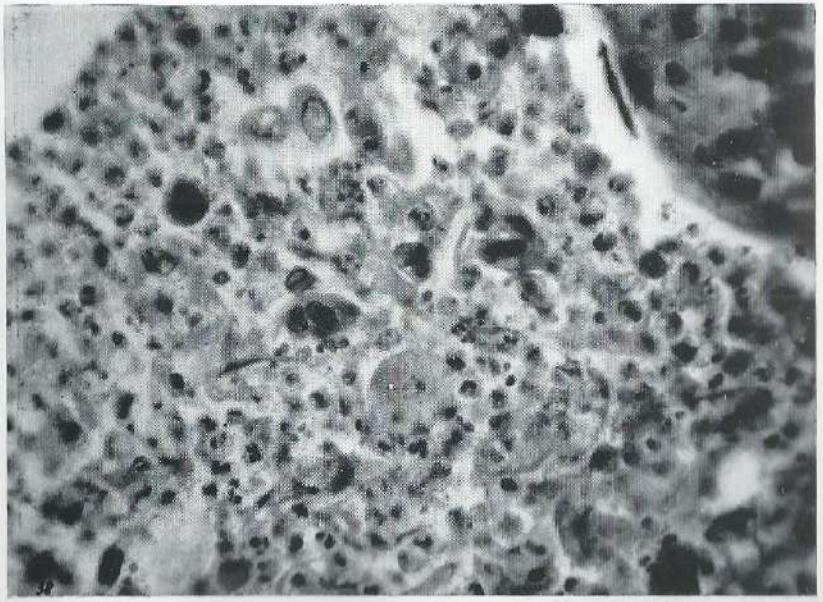


Fig. 38. The softened center of the epithelial pearl of Fig. 37. Several of these cells have the same appearance as those encountered in sputum smears.
Se., H.E. 400 ×

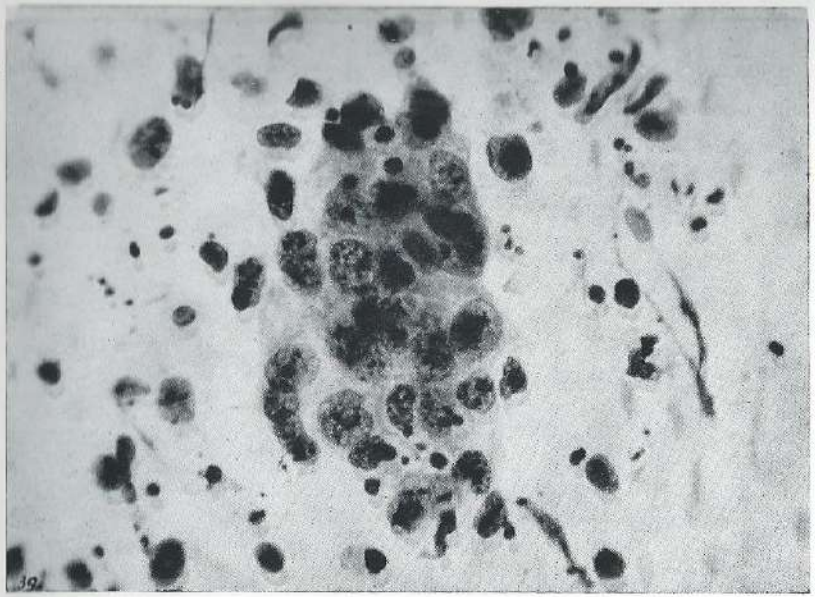


Fig. 39. Adenocarcinoma.
Cb., Pap. 400 ×

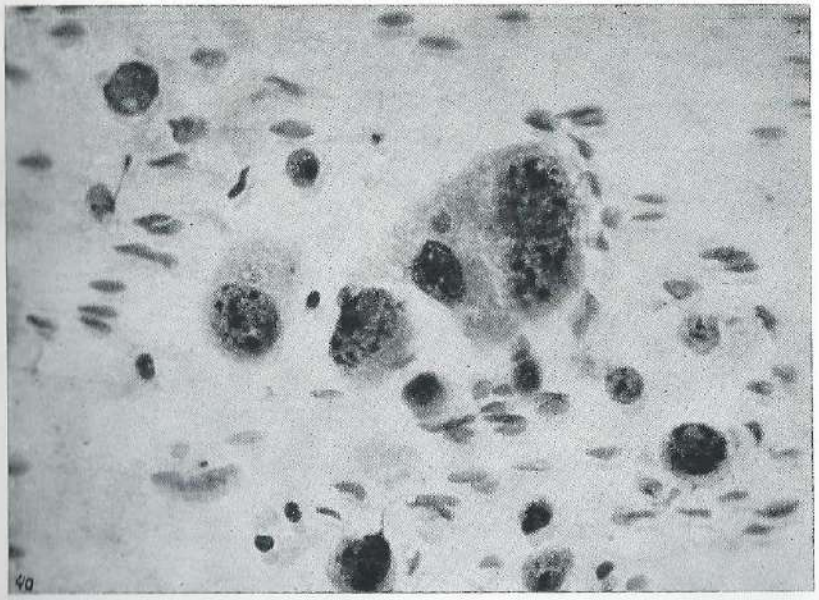


Fig. 40. Adenocarcinoma.
Co., H.E.A. 400 ×

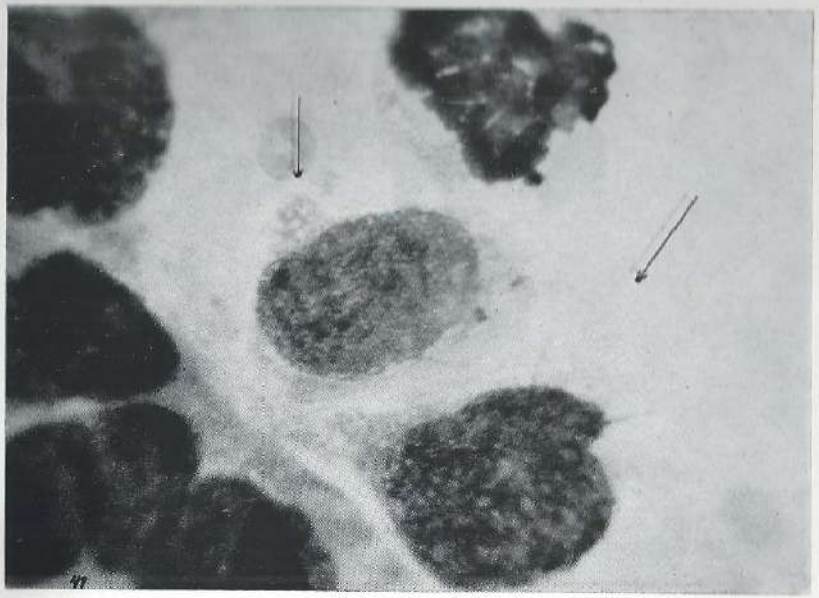


Fig. 41. Adenocarcinoma. The arrow indicates secretory vacuoles in the cytoplasm.
Lp., M.G. 1000 ×

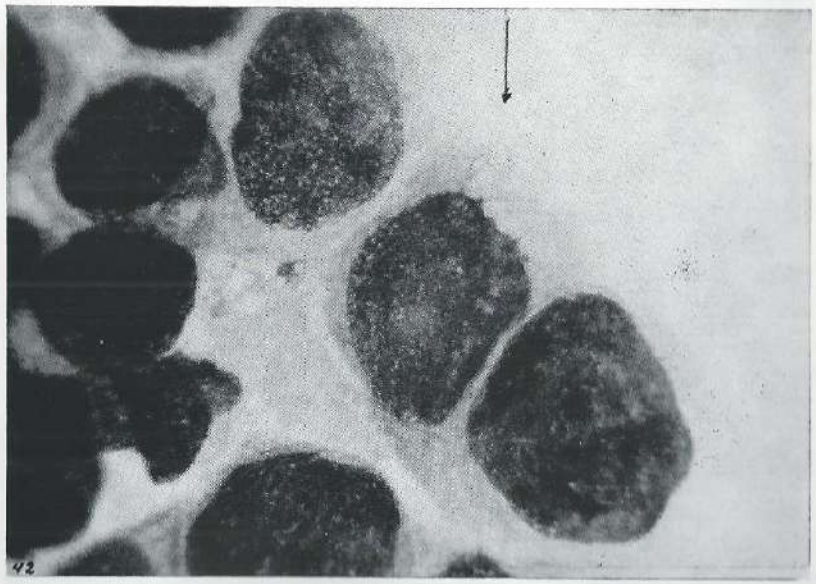


Fig. 42. Adenocarcinoma. The arrow indicates secretory vacuoles.
Lp., M.G. 1000 ×

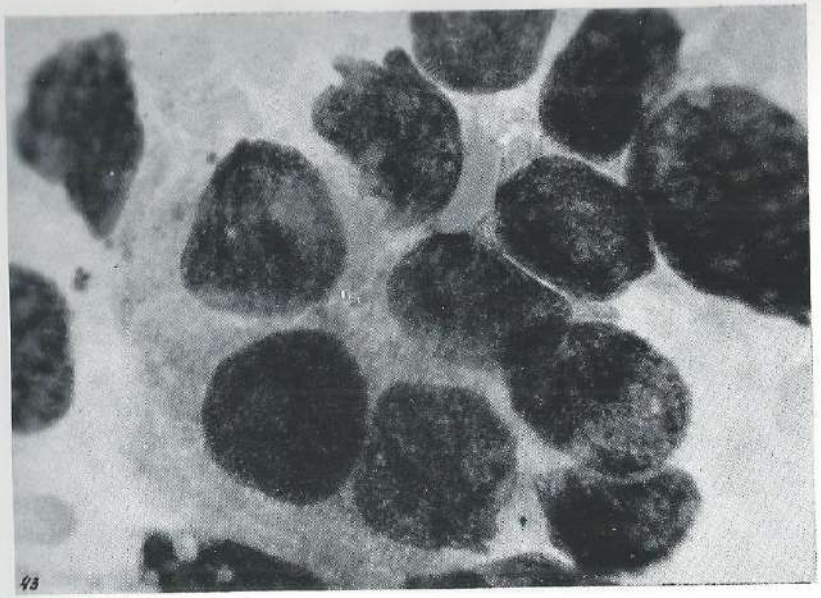


Fig. 43. Adenocarcinoma.
Lp., M.G. 1000 ×

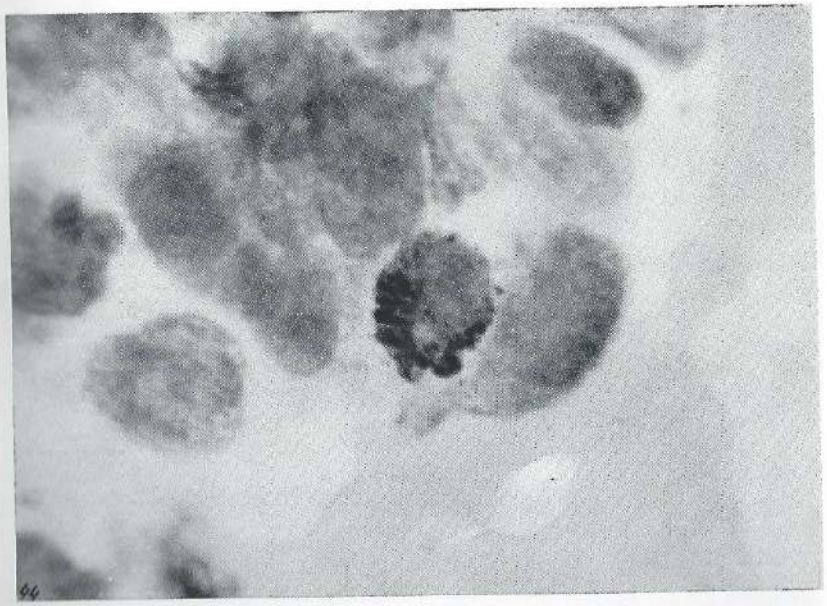


Fig. 44. Adenocarcinoma; from same specimen as that of Figs. 41–43. The secretory droplets, represented here in black, were bright red in the smear.
Lp., periodic acid-Schiff technique after Hotchkiss; alcoholic periodic acid solution. 1000 ×

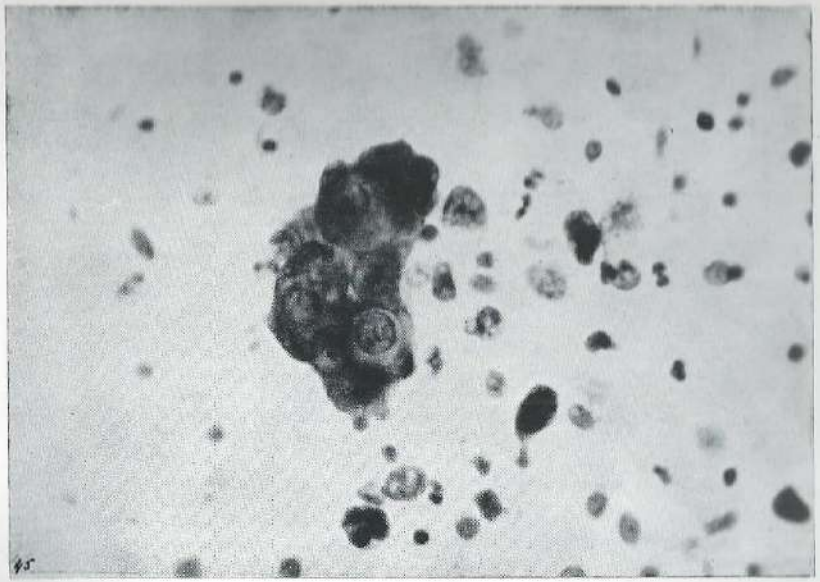


Fig. 45. Adenocarcinoma. The water-soluble mucin has spread throughout the cytoplasm and stains brightly red.
Cb., periodic acid-Schiff technique after Hotchkiss; aquous periodic acid solution. 400 ×

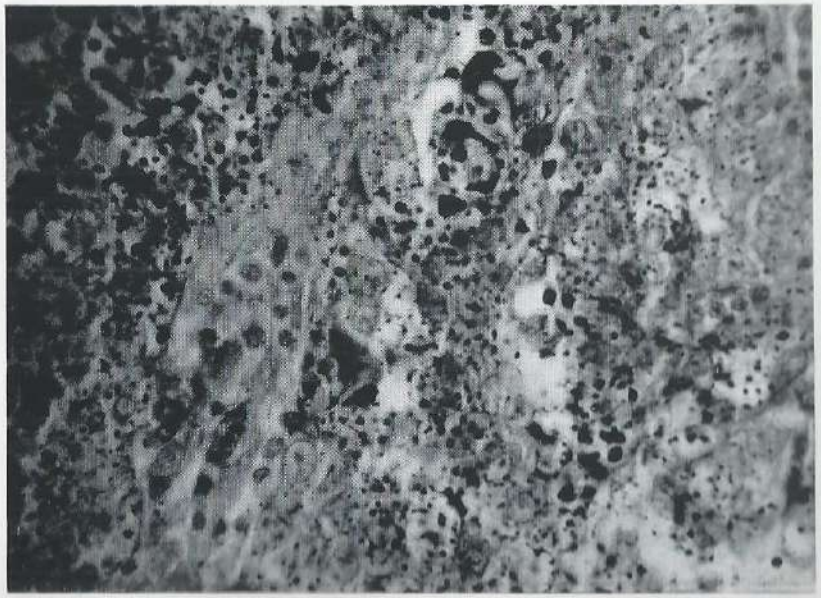


Fig. 46. Adenocarcinoma; from the same case as Fig. 45. In the lumen of the tubules and the cytoplasm of the cells are small and large mucin droplets.
Se., periodic acid-Schiff technique after Hotchkiss; alcoholic periodic acid solution. 400 ×

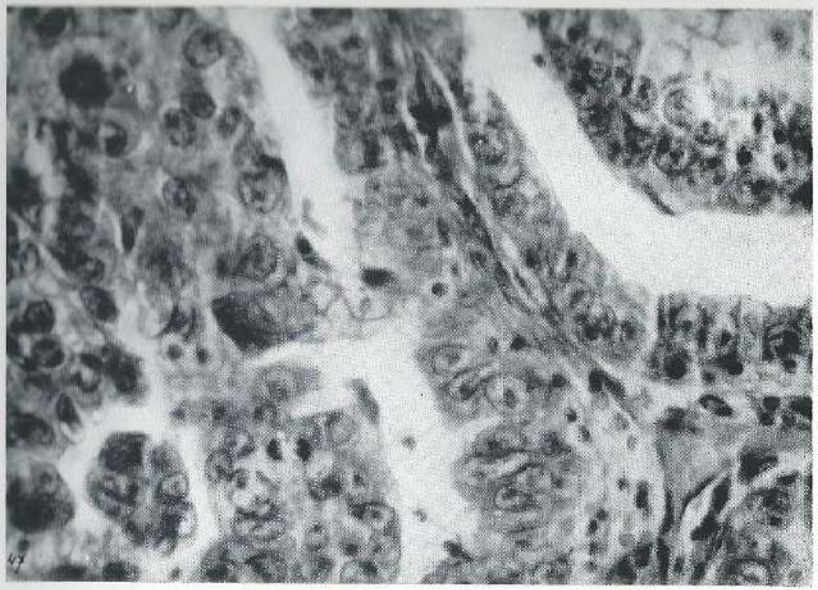


Fig. 47. Adenocarcinoma; from the same specimen as Fig. 45.
Se., H.E. 400 ×

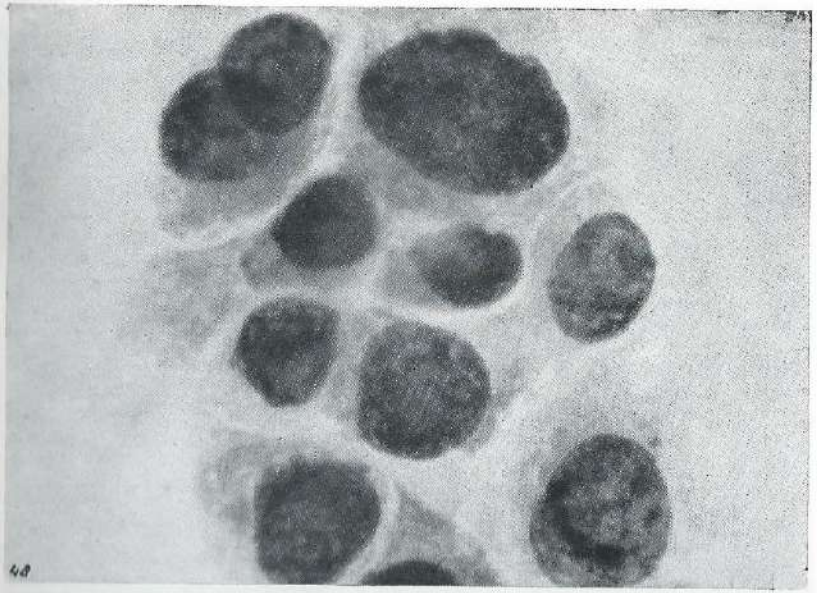


Fig. 48. Cylindrical cell solid carcinoma. The histologic section revealed only sporadic tubules.
Cb., M.G. 1000 ×

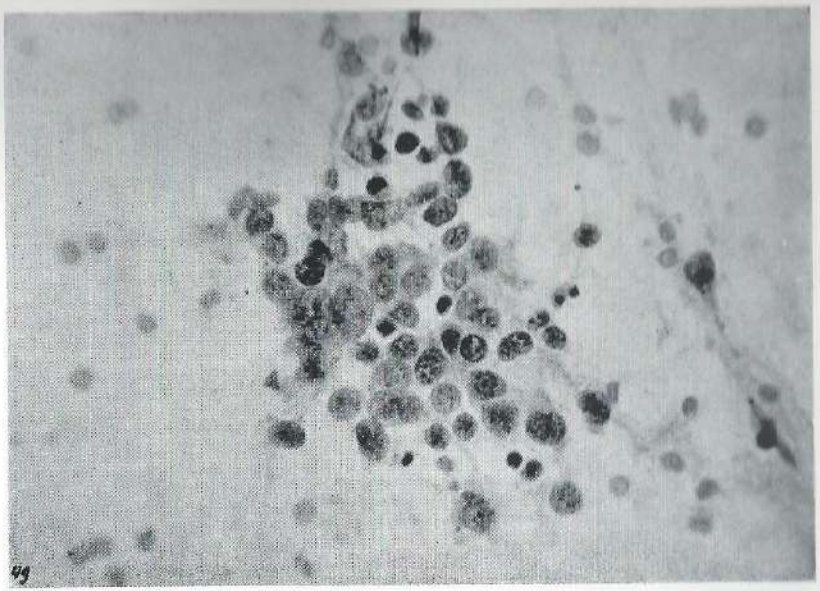


Fig. 49. Small cell solid carcinoma.
Cb., Pap. 400 ×

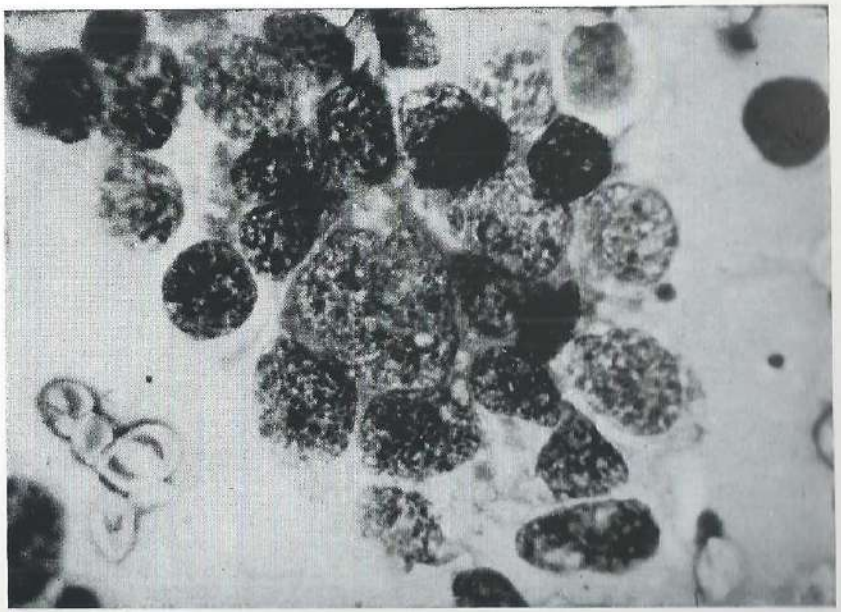


Fig. 50. Small cell solid carcinoma, with a typical fragmented chromatin structure and a narrow border of darkly staining cytoplasm.
Cb., M.G. 1000 ×

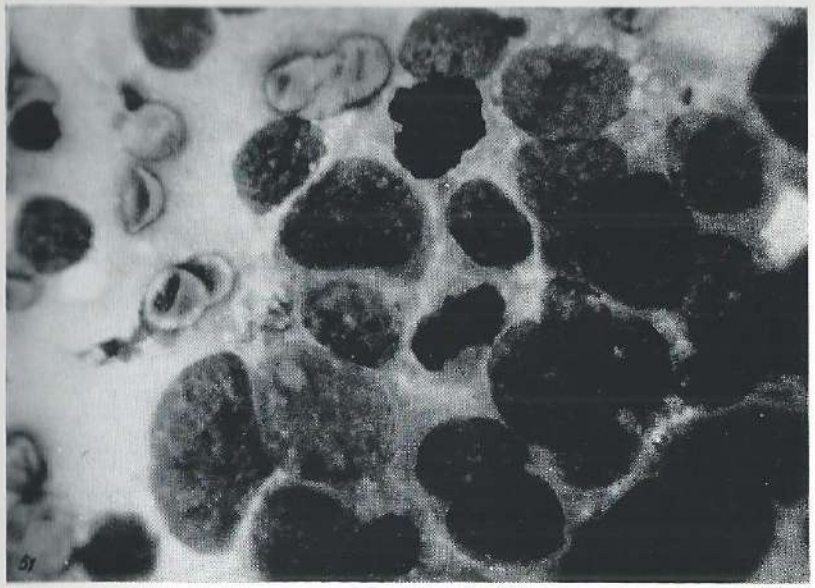


Fig. 51. Small cell solid carcinoma. Note two mitoses and chromophobe nucleoli.
Cb., M.G. 1000 ×

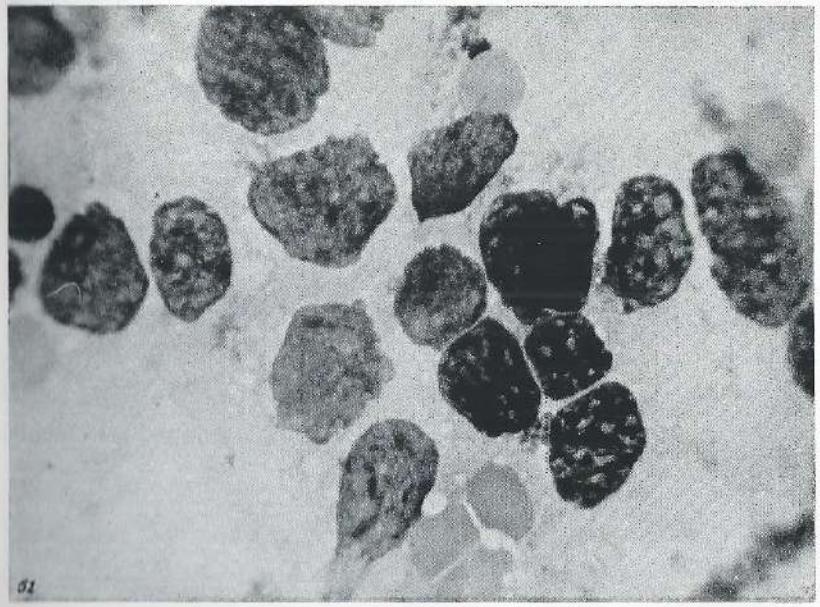


Fig. 52. Small cell anaplastic carcinoma; the bare nuclei have very irregular outlines.
Cs., M.G. 1000 ×

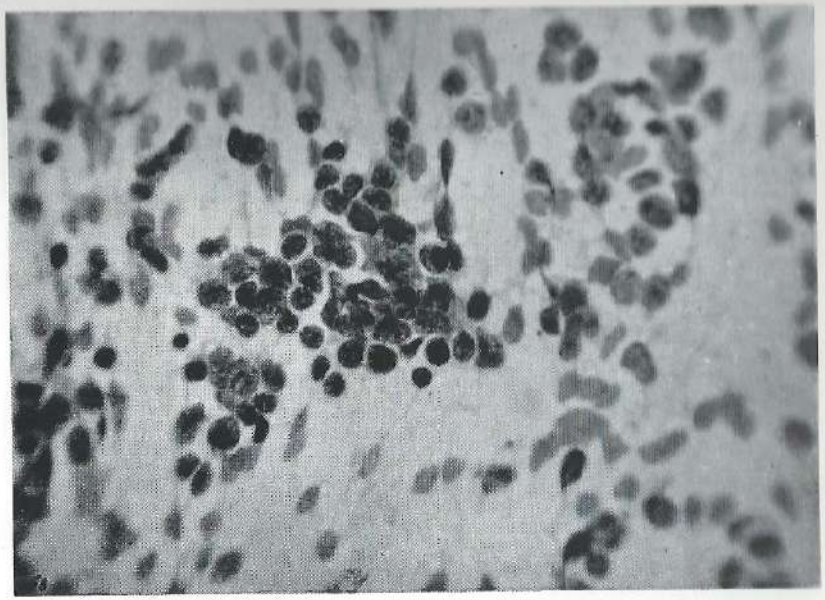


Fig. 53. Small cell anaplastic carcinoma.
Cs., Pap. 400 ×

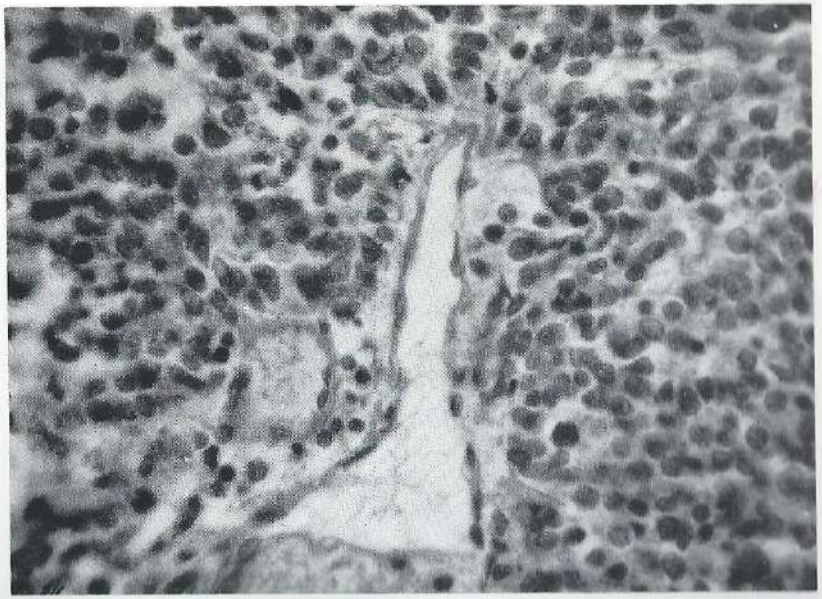


Fig. 54. Small cell anaplastic carcinoma.
Se., H.E. 400 ×

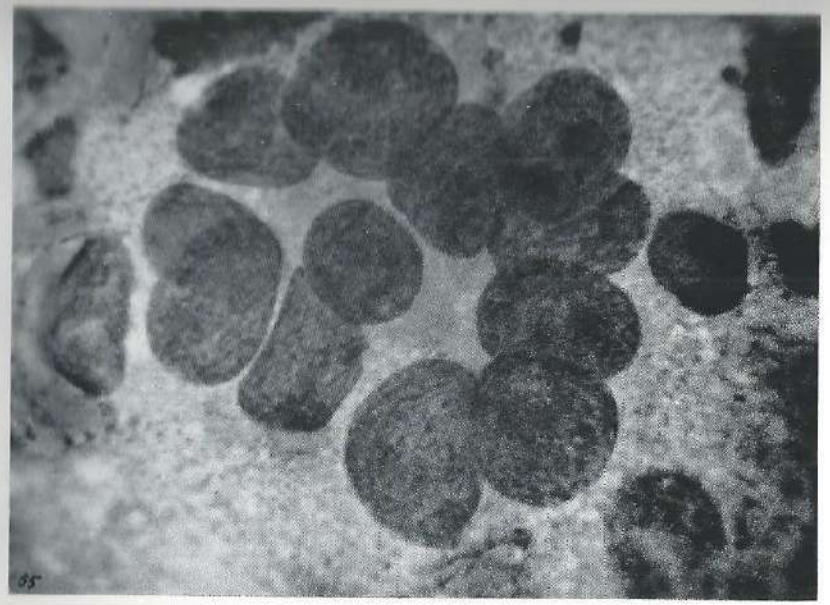


Fig. 55. Large cell anaplastic carcinoma; the cells originated from the anaplastic squamous cell carcinoma of Fig. 56. Bare nuclei, containing numerous polymorphic nucleoli (poorly reproduced on this photomicrograph), are embedded in a necrotic ground substance.
Co., M.G. 1000 ×

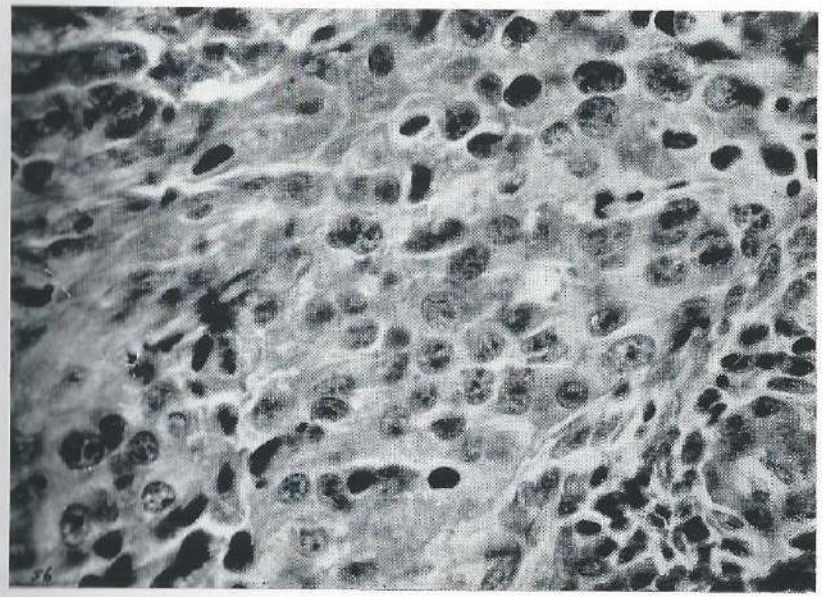


Fig. 56. Anaplastic squamous cell carcinoma.
Se., H.E. 400 ×

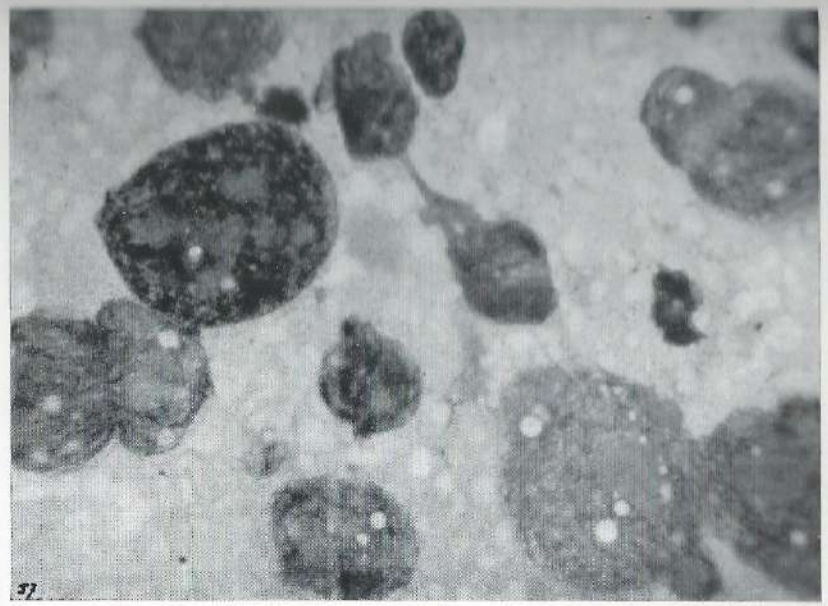


Fig. 57. Large cell anaplastic carcinoma; the smear also showed typical adenocarcinoma cells. There is nuclear vacuolation, and numerous polymorphic nucleoli are seen, which stained basophilic in the smear.
Co., M.G. 1000 ×

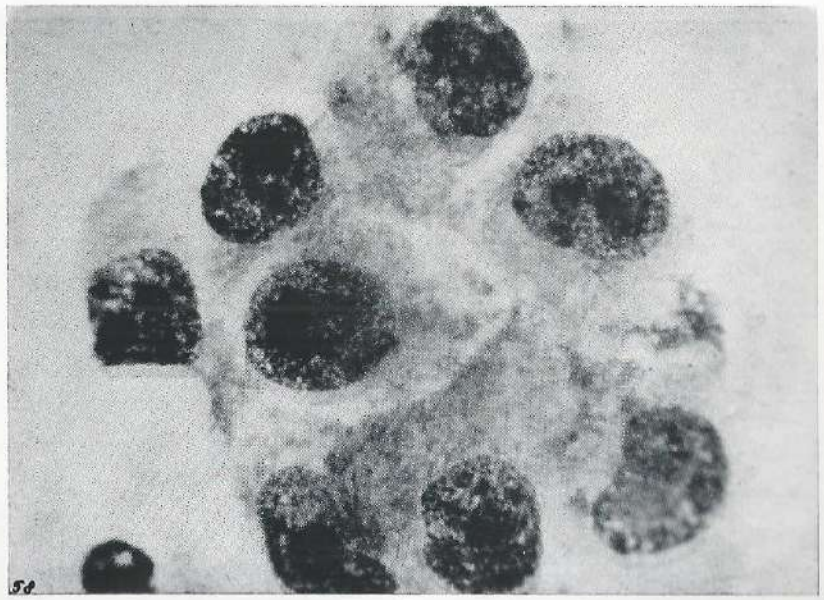


Fig. 58. Alveolar cell carcinoma. The cells have the shape of a flame or a circle sector and their eccentric nuclei contain one or two darkly-staining nucleoli. Compare the form and structure of these cells with those of Figs. 4 and 5.
Sp., M.G. 1000 ×

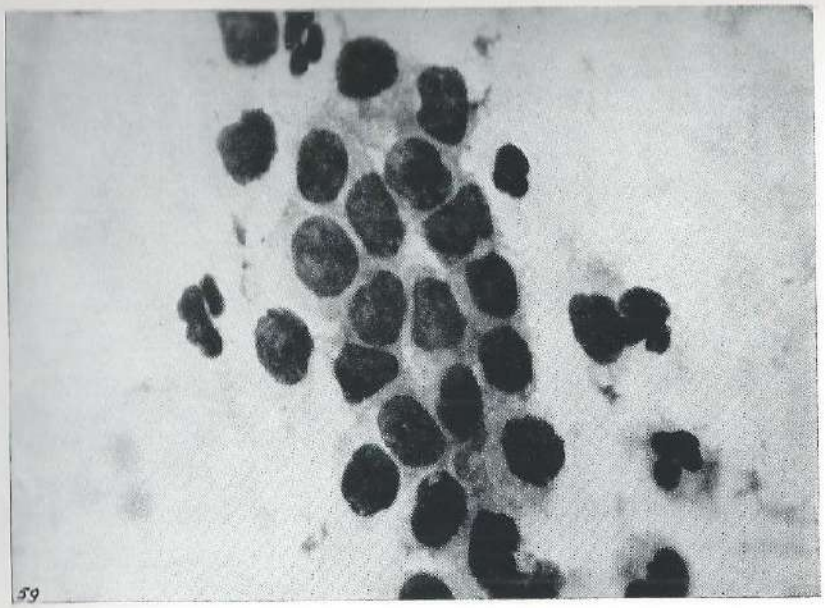


Fig. 59. Cells derived from a metastatic chorionepithelioma of the lung. They are small and contain a fairly large amount of cytoplasm and large nuclei. *Sp., M.G.* 1000 ×

STELLINGEN

I

Bij de beoordeling van de waarde van de cytologische maligniteitsdiagnostiek, dient men te onderscheiden tussen de betrouwbaarheid van de cytologische criteria van maligniteit op zichzelf en de betrouwbaarheid van een bepaald materiaal voor het stellen van zulk een diagnose.

II

Acute nephritis en polyarthritis acuta zijn gevolg-toestanden van infecties met streptococcus haemolyticus, waarvan type 12 en type 4 nephritis veroorzaken.
Deze feiten maken bacteriologisch onderzoek bij elke acute tonsillitis een eerste vereiste voor de prophylaxe van beide bovengenoemde ziekten.

III

De opvatting, dat een hiatus leucaemicus alleen bij de acute vorm van de myeloïde leucaemie voorkomt, is onjuist. Ook bij de chronische vorm is dit verschijnsel in meer of minder uitgesproken mate waar te nemen.

IV

Het is aannemelijk, dat langdurige ondervoeding van de moeder, gewicht en lengte van het kind bij de geboorte in ongunstige zin beïnvloedt.

V

De subicterische tint van een deel der lijders aan myxoedeem is te wijten aan een hypercarotinaemie, die het gevolg is van een gestoorde omzetting van carotine in vitamine A in de lever.

VI

De opvatting, dat maatregelen, die zouden lijden tot een zeer vroege diagnose van het mammacarcinoom, geen gunstige invloed zouden kunnen uitoefenen op de prognose na chirurgische behandeling, is onjuist. Daarbij is namelijk geen rekening gehouden met de mogelijkheid, dat een meer goedaardig carcinoom in de loop van de tijd evolueert in een kwaadaardige richting.

VII

Het valt zeer te betwijfelen of men bepaalde primitieve gedragspatronen, zoals die waargenomen kunnen worden bij lijders aan een schizophrene psychose, als archaïsch mag kenschetsen.

VIII

Het is gewenst, dat commissies in het leven worden geroepen, waarin clinici, pharmacologen en pharmaceuten zitting hebben, die zich ten doel stellen de therapeutische waarde en de toxiciteit van de vele moderne geneesmiddelen, die door de pharmaceutische industrie in de handel worden gebracht, critisch te onderzoeken.

IX

Het ware te wensen, dat tijdens de studie voor het propaedeutisch examen in de geneeskunde, de beginselen der medische statistiek werden onderwezen.

X

Het is dringend noodzakelijk, dat de studie van lichte ziektegevallen, alsmede de beginstadia van ernstige ziekten, georganiseerd wordt in instituten en klinieken, die beschikken over alle methoden en technische hulpmiddelen der moderne geneeskunde.