

Microorganisms in blood and tumour tissue from patients with malignancies of breast or genital tract

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Atypical microbes in blood and cancer tissue

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Abstract

Objectives

This study aims at looking for microbial growth in neoplasms and blood from patients with malignancies of breast or genital tract and to compare possible findings in both with those of healthy blood.

Method

Tissue and blood from 120 patients suffering from carcinoma of cervix and 50 patients suffering from breast cancer and in addition blood from 100 healthy volunteers were studied using Nomarski's interference contrast microscopy.

Result

Spherical soap-bubble-like forms, granules that move at great speed as well as long slender hyphae were observed. Some formations were suspected to be so-called L-Forms, others showed a growth-pattern similar to that of the pathogenic actinomycetes and fungi. These were most resistant to different kinds of chemical or physical influences.

Conclusion

Cell wall deficient, pleomorphic forms of microbes are found in both neoplasms and blood of cancer patients. They might be involved in the disease process.

Introduction

The answer to what causes cancer remains most elusive. Another question that may be as complex as the cancer cause, is the cancer provocation and incitement. Among the list of causes, microbes remain probably the oldest, but still a highly fascinating subject for investigations.

Studies on microorganisms in blood samples from both diseased and subjectively healthy persons have previously been reported^{1 2 3}. While many viruses have been found to be associated with tumour growth in both animals⁴ and human beings^{5 6}, bacteria and fungi are lesser known causative agents^{7 8 9}.

In the past decade a number of new and potentially significant microbes have been added to the list of more than 20 species inhabiting the human body that are significant pathogens in human disease¹. Primary microbiological analyses of cancerous human tissue have previously been conducted by us since these types of investigations have only been sparsely reported before^{10 11}. The use of the interference contrast microscopy technique was initiated in 1983 by one of the authors (E. E.), and investigations were primarily focused on vital blood samples from both control groups and patients diagnosed with cancer or other severe illnesses¹². Nomarski's interference contrast microscopy has made it possible to study the interaction of the tissue and its invaders with the least disturbance to the system under observation. While previously reported studies generally were concentrated on investigations of isolates, through blood- and tissue-cultures, the present study is focused on a direct examination of freshly obtained blood and tissue samples.

Materials and Methods

Peripheral blood as well as tumour tissue from 120 women suffering from carcinoma cervix and 50 women suffering from breast cancer were studied over a period of two years. Blood from 100 healthy female volunteers within the age group of 35 - 55 years served as controls.

Peripheral blood was obtained by puncturing the finger tip of a patient using an Autolet® finger pad puncture system after taking aseptic precautions. Strong antiseptics were avoided before pricking the finger to avoid affecting the fragile membranes of the erythrocytes. The drop of blood was collected on a sterile glass slide and a sterile coverslip was placed on the drop. The sample was allowed to spread evenly by capillary action in the space between the slide and the coverslip, care being taken to avoid application of pressure on the coverslip. The specimen slide was placed on the microscope and immediately studied.

Cancer tissue was freshly obtained from patients using aspiration cytology, biopsy and from surgical specimen. In the case of surgical specimen, tissue was taken for the present study as soon as the tissue was removed from the body without waiting for the surgery to be completed. Pieces of cancer tissue were taken from the centre of the specimen to avoid contaminating the microflora as far as possible. The tissue was placed on a sterile, dry glass slide and a sterile coverslip was placed on the specimen. The specimen was carefully pressed out to form a thin layer using an extra object slide placed on the coverslip. Leitz'® immersion oil was applied on the edges of the cover slip to prevent drying of the specimen. This technique enables a study of the sample for more than 100 hours under the microscope. The dynamic processes going on in the specimen under study can consequently be followed in real time. The initial microscopic examination was done immediately or within a maximum of three hours from the time of obtaining the specimen.

The Microscope used was a Leitz' Dialux 20® equipped with a modified UK condenser for darkfield, lightfield and interference contrast with a Plan-Floutar- objective, a binocular

phototube FSA and a 100 watt halogen lamp. All documentation was made using a Leitz' Vario Orthomat® automatic camera and Kodak® film. Video micrography was done using a Panasonic CD-20® Video camera attached to the microscope and a Panasonic video recorder. Video images were processed on line using a "Kramer®" active composite video processor and viewed on a Sony Trinitron® colour video monitor. All observations were initially made at 100xmagnification with the lightfield to obtain an overview of the specimen and then followed by the use of high magnification 1200x utilizing interference contrast.

Results

Small spherical irregular moving particles, less than 3 µm in diameter, were observed mainly in the plasma of blood from 68 of the 100 healthy individuals (*Figure 1*). In blood samples from patients, however, large microbe-like formations showing different morphological appearances were observed in the plasma together with the red and white blood cells (*Figure 2*).

Large colonies of small granules with sizes ranging from 0.5 to 3 µm were also found (*Figure 3*). These granules are quiescent when observed immediately after the specimen has been sampled. However, after a few hours, they eventually begin to move and subsequently, their activity increases dramatically. The granules can then spread out on the whole glass slide, and finally the whole blood sample is found teeming with these forms. In addition to this

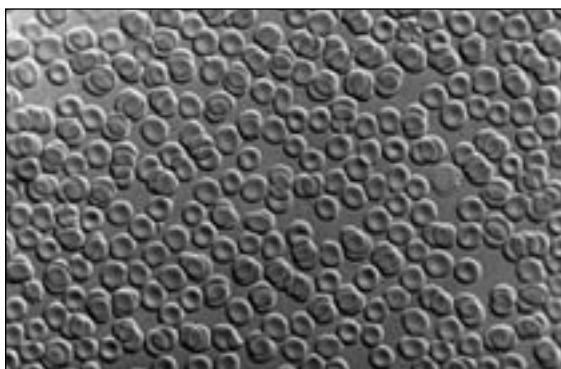


Figure 1. Normal Blood. The plasma is clear except for some occasional small granules.

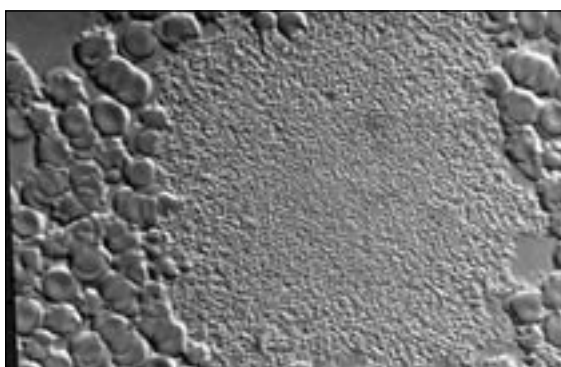


Figure 2. A roe-like accumulation of small granules. Note their varying sizes with the smallest particles in the central and biggest in the peripheral part of the colony.

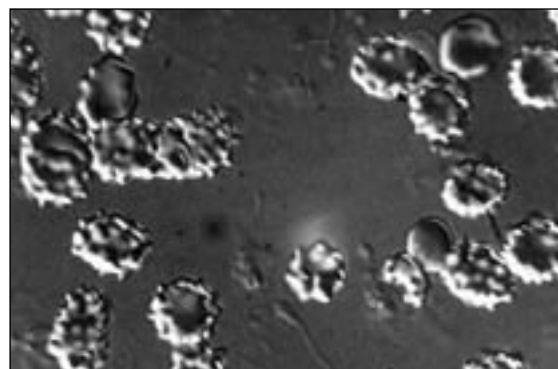


Figure 3. In the lower part of the picture a microbe-like formation, which shows a pronounced widening on its left upper end. The widened area sometimes seems to get stuck on the slide. Such a formation can move itself out of the visual field. Also note a principally similar structure in the upper part of the picture and small round particles spread out among the mostly aberrant RBCs.

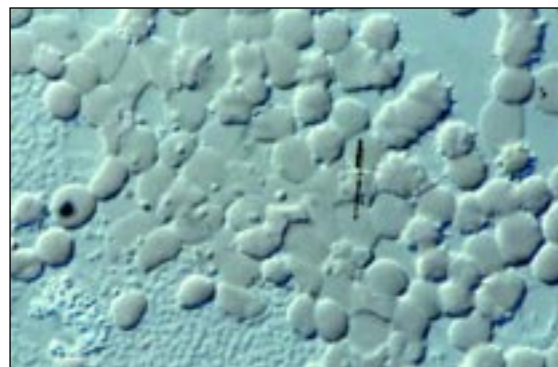


Figure 4. Granules in the periphery of a colony appear to come loose and go off into the surrounding plasma and mix with the erythrocytes.

uncontrolled activity, it has also been observed that these granules dissolve and disappear or even change their form and consequently develop into rod and dumbbell-like formations. Often, free granules at the edge of a colony show motion. After several hours, these granules disperse from the outer layers and move out among the surrounding erythrocytes (*Figure 4*).

When the specimen was kept under constant observation for a period of time it was found that the granules sometimes increased immensely in number. This could be indicated on the slide during the first week of observation. Two very special phenomena among these granules were noticed: a) a fusion of two granules into a new blob and b) a sudden explosion-like disappearance of these structures. Sometimes, the granules also developed into worm-like forms which were extremely active. In individuals with advanced cancer, large parts of the blood cells appeared to become totally destroyed when they interacted substantially with the granules (*Figure 5*). Long slender filamentous hypha-like structures could also be observed in some samples (*Figure 6*).

Spherical granules in the range of 2 to 10 μm in diameter were observed in cancer tissue (*Figure 7*). They were in constant motion in the fluid, but showed restricted mobility in the more solid parts of the specimen (*Figure 8*).

Microscopic studies of tissue samples from advanced cases of cancer also indicated much larger granules, sometimes attaining comparatively immobile giant sizes (*Figure 9*). Another observation utilizing the interference contrast microscopy was filament-like structures crossing



Figure 5. In this area of the blood-smear the red blood cells have assumed a pleomorphic appearance. In addition a tight scattering of active oscillating granules in different sizes are seen, which seem to be able to attack and destroy the blood corpuscles.

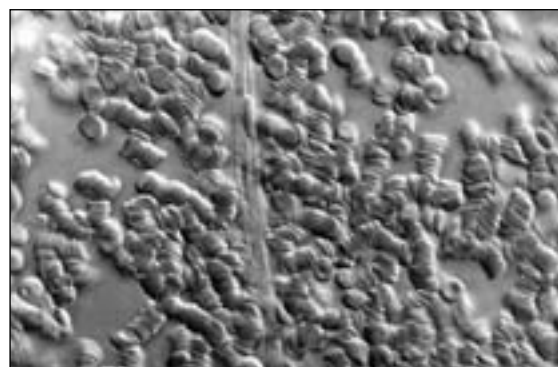


Figure 6. Note the hypha-like structure traversing the blood-smear.

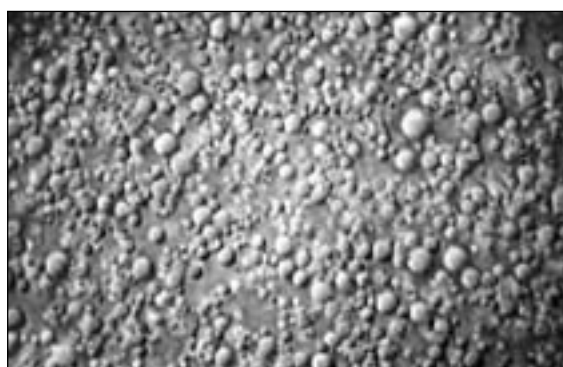


Figure 7. Granules in cancer tissue. Not a similar kind of particles as in the accumulations of granules in the blood but larger.



Figure 8. Granules in cancer tissue. In the upper part of the picture free oscillating particles. In the left lower part they are immobile and caught up by a cheese-like substance occurring in the cancer tissue and easy to press out to a thin layer between the slide and the cover-slip.

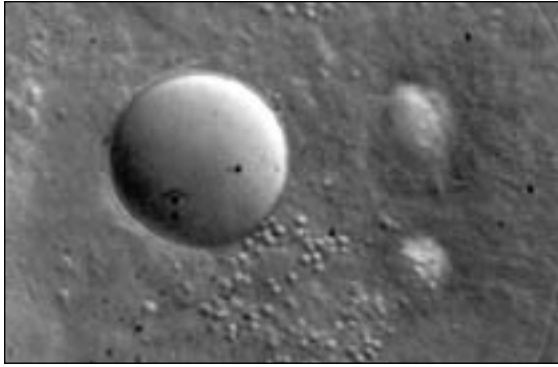


Figure 9. Granules in cancer tissue. Note the enormously large granule with a number of smaller ones around. In more advanced cancer, the number of these giant granules increases.

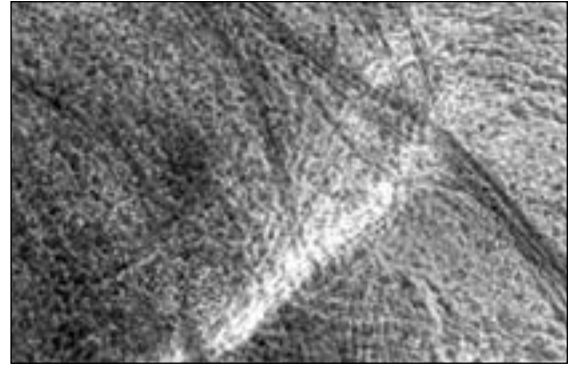


Figure 10. Filamentous structures in cancer tissue. Low power micrograph showing large bundles of long thread-like structures which are almost always to be seen in the more solid parts of a tumour tissue.

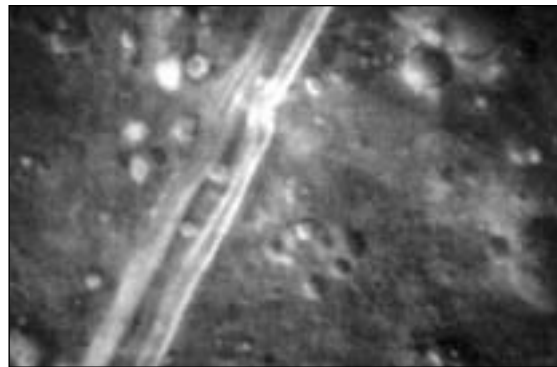


Figure 11. Hypha-like structures in cancer tissue. High power interference contrast study of the same tissue as in Figure 10 showing the long slender filaments. Also seen are the granular forms of varying sizes.

the solid parts of the specimen in all directions (Figure 10 and 11) and they appeared to be similar to those found in blood samples. In some instances, the granules described earlier were found along the filaments, seemingly connected to them. However, they did not show any mobility at all. The quantitative appearance of these observed microbial formations could reach close to 50% of the total volume of the tumour under investigation.

Alternate freezing to -10°C and thawing of the tissue for ten times or more did not alter the structure, quantity, mobility or the activity of these granules. Heating the tissue in a domestic microwave for 10 minutes using the power of 1200 watt did not seem to hamper their ability to oscillate. Antibiotics, antifungal and cytotoxic agents etc. failed to restrain the activity of these organisms.

Discussion

Microbes have been observed by many researchers in tumours and in blood as mentioned earlier in this paper. In order to research this, the interference contrast microscopy was selected by the authors as an appropriate technique. It also allowed the specimen to be observed for long durations without having to stain or fix it. This facilitates real time observations of dynamic changes without interfering the process or the substance.

Presence of tiny globular structures of less than $3\ \mu\text{m}$ in size in the blood of normal

individuals corroborates Enderlein's² observations of similar granules, which he termed "symbionts". The findings that they keep changing forms, from bacteria-like, to those of fungi and highly mobile worm-like forms, indicate a capacity to metamorphose. If observations were based on fixed and stained samples, the possibility for each stage or form to be interpreted as an entirely different organism remains high.

Since the days of Béchamp, polymorphism in microbes, despite many reports still remains an unpopular theme². Zopf¹³ in 1892 reported that "fission fungi, probably with some exceptions, are able to pass through different developmental stages". Further, Winkler¹⁴ in 1899 wrote that "Currently bacteriology holds the belief that each species of bacteria has only a certain very simple form ...and that it retains this form during its only mode of reproduction, which is by division into two...the slight change of form which happens during growth consists essentially of elongation, or shortening, or a local swelling. More remarkable deviations in form which are observed frequently are considered involution or degenerative". In contrast Winkler found that bacteria pass through stages with markedly different morphology.

The very few researchers who continued to work in line with the microbial cause for cancer at the beginning of the 20th century demonstrated that microbes had a remarkable pleomorphological tendency. Bunting¹⁵, studying Hodgkin's disease, isolated a pleomorphic organism that produced a picture of morbus Hodgkin when inoculated into animals. Mazet¹⁶, again studying this disease, isolated 26 strains of pleomorphic aerobes. His isolates were similar to those reported by the present authors in that they underwent transformations from granule stages to actinomycetes and yeast-like forms. Attempts to address the issue of malignancies facilitating microbes or caused by microbes were made by Diller et al.^{4 17}. They demonstrated that microbes were required for development of tumours. Diller's bacterium is a slow growing pleomorphic form that resembles both *Corynebacterium* and *Mycobacteria*. Nuzum's coccus¹⁸ isolated from human breast cancer is yet another example of a pleomorphic form that causes malignancies including primary epithelioma in man. Because of their high degree of pleomorphism and relative absence of cell walls, the organisms described were compared with "L-Forms" reported by Klieneberger-Nobel¹⁹, or a cell-wall deficient microorganism as defined by Dienes^{20 21}.

The organisms reported in our paper meet most of the criteria for atypical bacteria laid by Charache²² in several ways:

- a) Visualization of atypical forms directly from the clinical material.
- b) Failure of these forms to grow under conditions which would readily support growth of the parent organism (since routine blood culture reported negative, the authors consider this criterion to have been met).
- c) Ability of these organisms to survive in an osmotically controlled medium (the forms reported here have survived more than osmotic changes). However, while Charache's protoplasts are reported to disintegrate when heated, the forms reported by the authors did not exhibit a similar character.
- d) The clinical course consistent with bacteriological findings (the generally accepted view is that microbes are not expected to be found in the blood of patients suffering from breast cancer or cervix cancer). In carcinoma of the breast, where the growth has ulcerated, and in case of carcinoma cervix, tumour tissue may show bacterial contaminants. The authors have studied the organisms reported here in patients with different stages or severity of the disease and found a consistent pattern that may be considered as an appropriate clinical course.

Razin²³, studying the effect of various agents on mycoplasma, bacterial protoplasts, spheroplasts, and L-Forms reported that the mycoplasma and L-forms were much more resistant to lysis by osmotic shock and to alternate freezing and thawing than protoplasts and spheroplasts. The ability to survive alternate freezing and thawing and various conditions by the organisms

reported in this paper makes them similar to stable L-Forms.

Indications throughout the course of investigation suggest that these microorganisms undergo a development cycle. Löhnis²⁴ in 1916 observed "The development of the bacteria is characterised not by the irregular occurrence of more or less abnormal forms but by the regular occurrence of many different forms and stages of growth connected with each other by constant relations". Perhaps the phenomenon observed by the authors might subsequently be a case of completing cycle or only another case of metamorphoses, which is often found in biology.

It is surprising that many of the forms observed have been reported in various organisms. Löhnis²⁵ reviewed 1309 articles written between 1838 and 1918 and described many of the stages or forms:

- Globules or yeast-like forms in *Clostridium* by Ghon and Sachs and *Grassberger*, in *V. cholerae* by Maasen.
- Discharging cysts or giant round forms by Winogradsky in *Nitrosomonas* and in *Pneumococcus* by Artigalás.
- Filaments which branch or develop buds in *B. radicoila* by Conn, in *E. coli* by Matzuschita, in *Pasteurella pestis* by Albrecht and Ghon.
- Balloon forms with rhizoid sprouting in *M. leprae* by Lutz, in *V. cholera* by Fischer and in *S. rubrum* by Meirrowsky.
- Slender and long filaments or actinomyces-like stages in *M. tuberculosis* by Metchnikoff, in *M. leprae* by Meirrowsky, in *Pseudomonas* by Marx and Carpano and many others in many different bacteria. It is interesting to note that this stage have been seen in numerous species by many researchers.

By comparing the morphology and appearances of the microorganisms in samples from controls and cancer patients we have observed that there is a strong correlation between the morphology of the microbes and the present stage of cancer. This connection is not only found for different cancers or for acute infections, but it is also detected in samples from many chronic disorders such as multiple sclerosis, lupus, asthma etc^{12 21}.

Tumours could be a circumstance of a phenomenon representing a relationship between a growth product (the tumour), a soil (the tissue) and a factor causing a growth process (the infection), and growth can only continue if there is a nutritive soil in close relation to the growth product. We can maintain that nothing can grow in itself, as a growth-product cannot function as a nutritive soil to itself for its own growth. That could be an axiom. As growth throughout nature always reduces the nourishment contents of a soil towards poverty, the infectious growth process will gradually change and impoverish the tissues entirely according to the dominance of a growth process over a soil. Consequently, the capacity of a neoplasm to grow would mean that it is something distinctly different from the tissues within which it is growing. From a conceptual point of view it would also be impossible to defend the opinion that it is emanating from normal tissue cells that suddenly changed their behaviour, since the concept of growth implies a soil, which is something quite different from the growth product. The fact that plenty of granular forms are observed in tumours and great colonies of the same type in blood, allows for an assumption that the tumours represent only a fraction of the whole disease process in the body. Budding and multiplying locally, these granules may eventually create tumours in and at the expense of a tissue as substrate, changing its microstructure and metabolism and destroying it mechanically. The tumours may then be a nodal point from where dissemination of infectious granules out into the bodily fluids takes place, totally in accordance with the fact that spreading implies a focus.

Patients with growth processes in their tissues similar to those described in this paper, will often develop tumours, which then will become a very obvious part of their symptom pictures. Surgical excision or radiation here may be an equivalent of local treatment of a focus of

infection, thereby providing an explanation for the possibility of recurrence. Chemotherapy, in spite of acting on the body as a whole, may at times be ineffective. The microbe-like formations described above might help to explain this because the initiation and continuation of the malignant disease process might be due not only to so-called cancer cells of eukaryotic origin, where chemotherapy is the therapy of choice, but also to prokaryotic microbial activity, where this therapy is less or not at all effective.

Since the authors are clinicians more than microbiologists they wondered throughout the study as to why there is such a sparsity of reports and information in current textbooks in microbiology on pleomorphism and observations and findings such as those described above. Perhaps Löhnis' comment that "*standard textbooks contain photographs of the variants, never noticed because our eyes have been trained so very well not to see them*" is more true now than it was in 1921. Much needs to be done in evaluating and analyzing further the behaviour, culturability and the biochemical and immunological response of the forms observed. However, the authors believe that, if brought to notice, more results can be obtained from different centres, paving the way for a better understanding of the scourge that is cancer.

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