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REVIEW

Flow-Through-Cytophotometry

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Cytological methods have an established place in the diagnosis and the monitoring of treatment of malignant disease of the prostate and urothelium. The ease with which samples are obtained and the high accuracy of diagnosis has led to an increase in the number of these examinations. As the interpretation of cytological specimens is time-consuming and requires special knowledge of cell morphology, the attempts have been made to develop an automated method of examination.

Cancer cells differ from the normal cell population not only in morphology but also in biochemistry. An example is the difference in DNA and RNA contents (10, 15). Attempts to achieve automation in cytodiagnosis rely on these facts. Technical improvements such as the selective and quantitative staining of cell contents and the development of "flow-through-cytophotometry" or "pulse-cytophotometry" techniques have advanced the possibility of fully automatic cytodiagnosis.

Flow-through-cytophotometers work according to the following principle: A suspension of single cells is made from the tissue under investigation. Cells of interest are fluorochromated with special reagents and the prepared suspension is aspirated through the focus of a microscope photometer in a very thin jet. In the focal plane it is illuminated with UV-rays. By variation of the flowrate, the passage of up to a thousand cells per second is possible. In spite of this number of cells, the diameter of the nozzle is such that cells will pass through the focus individually. Light is emitted in linear proportion to the quantity of fluorescent material passing the beam. These signals are amplified by a photo multiplier, transferred to a multichannel analyser and stored according to luminous intensity. The results are printed as a histogram and analysed by a computer.

Depending on the type of cytophotometer, one or more cell substances can be measured selectively in a single passage. As already mentioned this is possible for the determination of DNA and RNA. Most earlier examinations measured only a single parameter - usually DNA.

Normal tissue predominantly consists of cells which are in the pre-synthetic phase (G1) of the cell cycle. They have diploid chromosomes (2c = 46 chromosomes) and a corresponding content of DNA. In a human cell this constitutes approximately 7 pg (13). In tissue which is not actively growing there are only a few cells past the S-phase (synthesis) with an increased DNA content. In the post-synthesis phase (G2) there exists a double set of chromosomes (4c) and a DNA content of about 14 pg per cell. Division of the tetraploid chromosomes during the following M-phase (mitotic-phase), returns the DNA content to 7 pg (Fig. 1). Therefore, when the DNA content of normal tissue is determined by flow-through-cytophotometry a histogram may be drawn (Fig. 2A). As most of the cells will have a diploid set of chromosomes, a Gaussian distribution with a high peak at 2c (7 pg per cell) is seen. The low second peak of the graph is produced by the few tetraploid cells (4c), which are found in normal tissue. Cells in S-phase will register on the histogram between 2c and 4c depending on their DNA concentration. The peak in

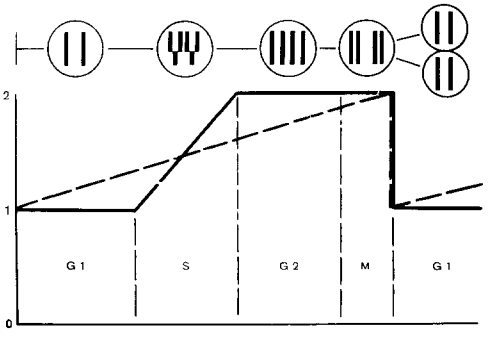

Fig. 1. Relative DNA (——) and protein (----) content of cells during the phases of the cell cycle

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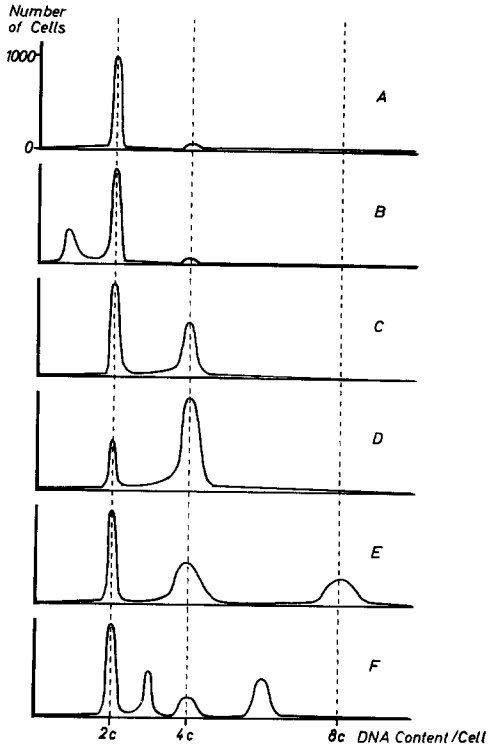

Fig. 2. Histograms of the relative DNA-content of cells in normal (A-B) and malignant tissues (C-F)

front of 2c is formed by cell fragments (Fig. 2B).

In growing tumours, i. e. tissue with an increased proliferation rate, the number of cells passing the S- and G2-phase, is increased. Therefore the histogram will show elevation of the graph between 2c and 4c and a higher peak at 4c (Fig. 2C). When the tumour is growing fast, the proportion of cells in the G 2-phase can exceed the number of cells in the G 1-phase (Fig. 2D). In tumours with a pathological division of their nuclei, polyploid or aneuploid stem lines can be found. Therefore, depending upon the number of cells beyond the G 2-phase, an extra peak may be seen at 8c. Cells showing pathological division and passing the G 1-phase, are registered together with cells of the diploid stem line, which are in their G 2-phase, at 4c (Fig. 2E). Extra peaks outside the duplication content of 2c are formed by aneuploid nuclei (Fig. 2F).

Initial attempts to measure DNA by pulse cytophotometry were confronted by difficulties in the preparation of the cell material. These problems are now generally solved and standardised methods are now available. The fluorochromation of DNA is produced by ethidiumbromide, or acridine-orange (1, 6).

Following standardisation of techniques, much research work has been carried out concerning DNA distribution in normal and pathological tissue and with determination of cell division in different organs. The results show, for instance, that this method is suitable for the determination of the degree of synchronisation of cell cycles during cytostatic or radiation therapy (5, 12, 18). As the effect of cytostatics is limited to certain phases of cell division, it is one of the aims of oncology to induce synchronisation of the division phases. Pulse cytophotometry has proved to be of value in determining the time of maximum synchronisation of cell division. Improved timing of the cytostatic therapy could lead to better results and this approach has already been followed in the therapy of leukaemia and testicular tumours (3, 7).

Another field of application for pulse-cytophotometry is gynaecology, where it may be used in the diagnosis of cervical cancer. In spite of some problems, automated cytodiagnosis in gynaecology shows encouraging results (14). One problem is the difficulty resulting from hormone induced changes of the histograms (13). On the other hand it is possible to follow these changes of cell kinetics by cytophotometry. A further field of application is the investigation of fluid obtained from ovarian cysts or the pouch of Douglas in cases where malignant ovarian tumours or peritoneal metastasis are suspected (2, 8).

Good results have been achieved in cases with stomach cancer (19), malignant melanoma (9) and bleeding from the nipple (2). In another study the pulse-cytophotometry of cells obtained by needle or open biopsy of the breast showed favourable results.

PULSE-CYTOPHOTOMETRY IN UROLOGY

In the urological literature there are only a few publications concerning pulse-cytophotometry of urine. One group (11) has determined DNA and RNA simultaneously and has developed methods of staining RNA in benign and malign cells differently. This method has already produced encouraging results but more research is necessary.

Another group (4) compared the reliability of cytology and pulse-cytophotometrical determinations of DNA in 130 urine specimens. Cytophotometry produced twice the number of incorrect results compared with cytology. It is presumed that nuclear atypia is responsible for this discrepancy. However, most investigators believe that with improvement of preparation techniques, flow-through-cytophotometry of urine can become a supplement to cytology in the routine diagnosis of bladder cancer (16). Reviewing our own results we have also come to the conclusion that within certain limits pulse-cytophotometry of prostate and urine makes the automation of cytology possible (17, 21). Examining cells obtained from the prostate by fine needle biopsy, good correspondence between pulse-cytophotometrical determinations of DNA and conventional cytology and histology has been achieved. Pulse-cytophotometry of urine has proved to be less reliable than cytology in our studies. Nevertheless, we are optimistic and believe that by further development of the method

and by examining more parameters, it may be possible to decrease the number of errors.

The potential applications of pulse-cytophotometry are numerous. Likely uses in urology are:
1. Automated cytological prescreening for carcinoma of the prostate and the urinary tract.
2. Follow-up of cancer of the prostate and the urinary tract.
3. Grading of malignant tumours of the urinary tract.
4. Determination of synchronisation of cell division for optimum effect of cytostatus or radiation.
5. Experimental and clinical sperm research.
6. Examination of proliferation kinetics in different hetero transplantations in experimental urology.

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