turized and up-dated to accomodate a central straight dosage line in the surface of the valve block. One block contains ten valves in the required combination. The most important part of the sequencer is the control program for the PC. This program might be compared to a text-editor. The user can write his own text (in this case, his own degradation program). Of course, he also can use a general degradation program distributed together with the sequencer.

The program has two main parts: one is the EDIT-mode for writing or changing a user program; the second part is to run the program on the sequencer. Even during the run, it is possible to modify the program, so as to change the activated valves or to modify the time of activation.

The complete program is controlled by function keys and easy to learn.

In conclusion, the control program for the sequencer is very flexible and comfortable to use, especially to adapt the program to research with new chemistry for degradation or conversion.

1 Frank Reimann, F. & Wittmann-Liebold, B. (1986) in *Advanced Methods in Protein Microsequence Analysis* (B. Wittmann-Liebold, B., Salnikow, J. & Erdmann, V.A., eds.) pp. 118–125, Springer-Verlag, Berlin.

F. Reimann, Fa. Dr. Knauer GmbH, Hegauer Weg 38, D-1000 Berlin 37.
B. Wittmann-Liebold, Max-Planck-Institut für molekulare Genetik, Ihnestr. 73, D-1000 Berlin 33 (Dahlem).

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Cloning in Procaryonts

No abstract received.

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Karenzin – a Hormone-Dependent Secretory Protein of the Rat Seminal Vesicle with Actin-Binding Properties

In sexually inactive male rats the residual secretion of the seminal vesicles contains one prevalent protein with a molecular mass of 49 kDa which we have termed karenzin (SVS II). This protein is synthesized and secreted in an androgen-dependent manner[1].

Several biochemical and cell biological properties of the polypeptide could be elucidated using gold-labeled reagents which were applied to transblots of the protein on nitrocellulose strips after SDS-polyacrylamide gel electrophoresis. Colloidal gold-particles bound to the marker substances were visualized using a silver intensification method (IGSS-method[2]).

By use of gold-labeled lectins we have shown karenzin to be a glycoprotein with terminally bound L-fucosyl, D-mannosyl or glucosyl and D-galactosyl residues.

The specificity of polyclonal rabbit antibodies against karenzin was visualized through gold labeling of the primary antibody which was used in immunoprint-analysis. In addition it could be clearly shown that the lateral prostate produces an identical protein.

Gold-labeled DN'ase I, the specific binding of which to actin is well known, similary binds to karenzin as well as to karenzin fragments (molecular mass 17 and 20 kDa, respectively) that are formed by limited proteolysis through V8-protease.

Karenzin binds to the filament protein actin and precipitates it from respective extracts, thereby depolymerizing F-actin. The stoichiometric relationship is 1 : 2. Using the immunocomplex of karenzin and gold-labeled anti-karenzin applied to nitrocellulose-immobilized actin or actin fragments formed by V8-proteolysis we have shown that

(i) karenzin has a high affinity to sceletal- and heart-muscle actin and
(ii) presumably binds to the C-terminus of this structural protein.

1 Aumüller, G. & Seitz, J. (1985) *Histochem. J.* **18**, 15–23.
2 Moermans, M., Daneels, G. & De Mey, J. (1985) *Anal. Biochem.* **145**, 315–321.

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G. Valet

New Developments in Cellbiochemical Analysis by Flow-Cytometry

The investigation of biochemical regulations in malignant tumors or in the immune and hematopoietic system is difficult with normal biochemical methods due to the complexity of the cellular composition of these tissues. Cell culture systems can replace the study of native tissues only in part since the in-vivo composition of the many different cell types of a fresh tissue sample is quickly lost in culture and it is not possible to adequately compose the tissue architecture from established cell lines.

Flow-cytometric methods offer a new approach to this problem. Biochemical and microscopic analysis at the single cell level are combined with high speed of analysis (e.g. 1000 cells/s) in a flow-cytometer. Several biochemical or biophysical parameters of each cell can be simultaneously measured. Parameters for measurements are either cell *constituents* such as antigens[1,2], hormone receptors, lectin receptors[3,4], DNA[5], RNA, total cellular protein, lipids, electrical surface charge density[6] or in recent years increasingly the measurement of *functional* biochemical properties of vital cells. Various biochemical pathways of vital cell can be probed. Hydrolytic enzymes such as esterases, phosphatases, proteases or peroxidases[7,8] permit the estimation of the internal catabolic potential of cells. The measurement of H_2O_2 production, free radical formation, metabolic burst activity[8] and glutathion levels[9]

give information on the oxido-reductive state. The determination of transmembrane and mitochondrial potentials[10], of intracellular calcium[11,12] and intracellular pH[13,14] are correlates for the activation and metabolic state of cells.

Practical applications of flow-cytometry are the identification and characterization of cellular subpopulations in tumors[1,2] or in the immune and hematopoietic system[15]. The functional characterization permits to analyse reactions of cellular systems to external substances and influences such as irradiation with ionizing radiation[16], cytostatic drugs[17–19], hormones, metabolites etc. An interesting possibility is the measurement of the amount of intracellular enzymes e.g. elastase or myeloperoxidase by fluorescent antibodies. The functional activity of these enzymes can then additionally be determined with fluorogenic substrates in a separate experiment on an aliquot of vital cells.

The efficient data analysis is an important requirement for flow-cytometric work. We have developed the DIAGNOS 1 program system which calculates the results and databases them. It furthermore has for the first time in flow-cytometry selflearning capacities and is capable of automatically finding minor biochemical differences of cells by a multifactorial analysis of several cell parameters[20]. The speed of sample measurement and sample preparation in microtiter plates can be increased by using a laboratory robot system[21].

1 Valet, G., Kahle, H., Wirsching, R., Liewald, F., Demmel, N., Rübe, Ch. & Warnecke, H.H. (1987) in *Endokrin aktive maligne Tumoren* (Engelhardt, D. & Mann, R., eds.) pp. 11–20, Springer, Berlin.
2 Valet, G., Rüssmann, L. & Wirsching, R. (1984) *J. Clin. Chem. Clin. Biochem.* **22**, 935–942.
3 Malin-Berdel, J., Valet, G., Thiel, E., Forrester, J.A. & Gürtler, L.A. (1984) *Cytometry* **5**, 204–209.
4 Siegert, W., Mönch, T. & Valet, G. (1980) *Exp. Hematol.* **8**, 1173–1182.
5 Valet, G., Fischer, B., Sundergeld, A., Hanser, G., Kachel, V. & Ruhenstroth-Bauer, G. (1979) *J. Histochem. Cytochem.* **27**, 398–403.
6 Valet, G., Bamberger, S. & Ruhenstroth-Bauer, G. (1979) *J. Histochem. Cytochem.* **27**, 342–349.
7 Malin-Berdel, J. & Valet, G. (1980) *Cytometry* **1**, 222–228.
8 Burow, S. & Valet, G. (1987) *Eur. J. Cell Biol.* **43**, 128–133.
9 Treumer, J. & Valet, G. (1986) *Exp. Cell Res.* **163**, 518–524.
10 Hasmann, M. & Valet, G. (1987) *J. Cancer Res. Clin. Oncol. Suppl.* **113**, 30.
11 Valet, G. & Raffael, A. (1985) *Naturwissenschaften* **72**, 600–602.
12 Raffael, A. & Valet, G. (1987) in *Proc. Int. Symp. Clin. Cytometry Histometry, Elmau 1986*, Academic Press, London, in press.
13 Valet, G. & Raffael, A. (1984) in *Cyto-P-Check*, pp. 1–21, Paesel GmbH, Frankfurt.
14 Valet, G., Raffael, A., Moroder, L., Wünsch, E. & Ruhenstroth-Bauer, G. (1981) *Naturwissenschaften* **68**, 265–266.
15 Valet, G. (1984) *Blut* **49**, 83–90.
16 Valet, G. & Kahle, H. (1986) in *Biological Indicators for Radiation Dose Assessment* (Kaul, A., Dehos, A., Bögl, W., Hinz, G., Kossel, F., Schwarz, E.R., Stamm, A. & Stephan, G., eds.) vol. 2/86, pp. 211–223, Schriftenreihe des Bundesgesundheitsamts, MMV-Medizin, München.
17 Valet, G., Warnecke, H.H. & Kahle, H. (1984) *Blut* **49**, 37–43.
18 Valet, G., Warnecke, H.H. & Kahle, H. (1984) in Cyto-P-Check, pp. 22–30, Paesel GmbH, Frankfurt.
19 Warnecke, H.H., Kahle, H. & Valet, G. (1985) in *Aktuelle Onkologie* (Nagel, G.A., Sauer, R. & Schreiber, H.W., eds.) vol. 16, pp. 101–107, Zuckschwerdt, München.
20 Valet, G. (1987) in *Proc. Int. Symp. Clin. Cytometry Histometry, Elmau 1986*, Academic Press, London, in press.
21 Manta, V., Treumer, J. & Valet, G. (1987) in *Proc. Int. Symp. Clin. Cytometry Histometry, Elmau 1986*, Academic Press, London, in press.

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Amino-Acid Sequence Analysis of Proteins Separated by One-and Two-Dimensional Gel Electrophoresis and Electroblotted on Polybase-Coated Glass-Fiber Sheets

We have previously demonstrated that proteins can be electroeluted from polyacrylamide gels and immobilized on polybrene-coated glass-fiber sheets. These sheets serve as support for gas-phase sequence analysis and complete acid hydrolysis of the bound proteins[1]. Here, we report on the use of different polybases in combination with various types of transfer buffers. Most efficient protein elution out of gels and the highest amount of protein bound per area unit of glass fiber is obtained in the following conditions: electroelution during 6 h in 50mM Tris/borate pH 8.3 buffer and immobilization on Whatman GF/C glass fiber sheets coated with poly-(1-methyl-4-vinylpyridine). Protein detection is carried out by dipping the dried glass-fiber blots for less than 2 s in a solution of 1 mg fluorescamine in 1 *l* of acetone.

The protein glass-fiber blotting was used so far to determine the NH_2-terminal sequence of more than 100 proteins or protein fragments:

(i) analysis of gel-separated subunits of a multisubunit enzyme complex which was isolated by immunoaffinity using an antibody directed against one of the subunits,
(ii) analysis and confirmation of NH_2 terminal sequences of recombinant proteins,
(iii) fast mapping of epitopes and functional domains,
(iv) to provide amino-acid sequences from which specific DNA-probes can be obtained in order to identify and to isolate corresponding genes.

Recently, we have applied this procedure for analysis of two-dimensionally separated proteins. Fifteen different proteins from one single gel were isolated in

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