thymus (fig. D) and lymph node (fig. E), and with the cytoplasm of approximately 20% of nucleated cells of bone marrow (fig. F).

Discussion. The capacity of anti-Br-MRBC autoantibodies to react with antigens in multiple organs may be seen in the context of polyreactivity of monoclonal IgM autoantibodies. Both in mice and humans, in fact, most monoclonal IgM autoantibodies described so far, isolated from normal individuals or from patients and animals with autoimmune disorders, when studied in detail, turned out to be polyreactive[10,11] and often to react with antigens in a number of different organs[12–16]. The biochemical basis of multiple organ-reactivity of monoclonal autoantibodies seems to be the presence of common antigens or epitopes in different organs, or similar, but not identical, epitopes to which the autoantibody can structurally accommodate[17]. Although the significance of multiple organ-reactive IgM autoantibodies is still undefined, the constancy of finding these autoantibodies suggests that they are a common feature of the normal B cell repertoire. A possible answer to the question of the role of these autoantibodies might come from the recent discovery of the 'Ly-1 B' cells. In normal and autoimmune NZB mice, in fact, these cells constitute a functionally distinct B cell subpopulation, bearing surface IgM and IgD and Ly-1 B antigens[18,19]. The Ly-1 B cell subpopulation is thought to play some important role in autoimmunity because it contains virtually all the cells that spontaneously secrete IgM autoantibodies to several commonly studied autoantigens, such as Br-MRBC, thymocytes and single-stranded DNA, but only minimal numbers of cells that give an IgM response to exogenous antigens[19]. One possibility could be that Ly-1 B cells produce autoantibodies to aged or dead cells or tissues expressing 'hidden' autoantigens, in order to favor their clearance from the body. If this is the case, polyreactivity of these autoantibodies would endow the immune system with an economy for antigen recognition.

Multiple organ-reactivity, however, is not only restriced to IgM autoantibodies. It has been, in fact, recently reported that anti-idiotypic antibody to a multiple organ-reactive monoclonal IgM autoantibody can induce IgG antibodies showing the same pattern of tissue reactivity as the IgM monoclonal autoantibody[20]. This raises the possibility of a further mechanism to explain the occurrence of an IgG-mediated autoimmune response to certain tissues or organs; for example, if a tissue is injured, B lymphocytes committed to producing autoantibodies to epitopes in multiple organs, upon exposure to any of these epitopes, could be triggered to the production of IgM autoantibodies which are necessary for the clearance of the injured tissue; this response will be followed, according to Jerne's network theory, by an anti-idiotype response that, in turn, will trigger an anti-anti-idiotype response. This could be directed not necessarily only to the tissue that was initially injured and that initiated the cascade, but also to any tissue expressing that epitope. Thus, the existence of 'normal' multiple organ-reactive autoantibodies could turn to be one of the key mechanisms for triggering autoimmune responses.

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Changes in intracellular pH and cell volume during the early phase of DMSO-induced differentiation of Friend erythroleukemia cells[1]

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Summary. Changes in intracellular pH and water volume were measured after treatment of Friend erythroleukemia cells with 1.5% DMSO. It was found that a continuous decrease in pH_i occurred, beginning 1 h after induction and a decline in pH_i of 0.18 was measured after 9 h. In addition a decline in cellular water volume, of 12% only 15 min after induction, and 23% after 9 h, was observed.

Key words. Friend cells; differentiation; intracellular pH; cell volume.

The intracellular pH appears to play an important role in the regulation of cell growth. An elevated pH_i corresponds with mitotic activity in various mammalian cell systems[4,5]. Treatment with mitogens leads to a quick alkalinization of the

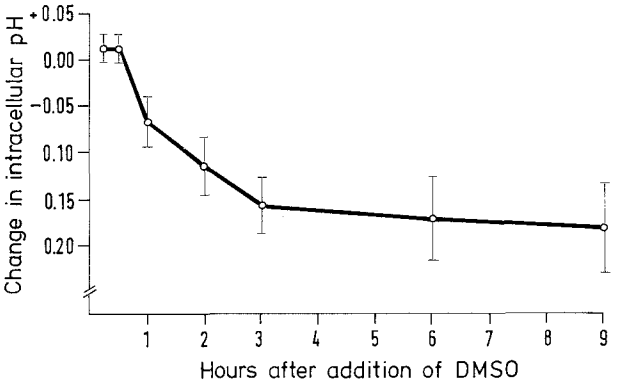

Figure 1. Changes in intracellular pH during the early phase of DMSO-induced differentiation of F4–6 cells. The pH_i scale represents differences in pH_i relative to the control. Means of three experiments in triplicate ± SE.

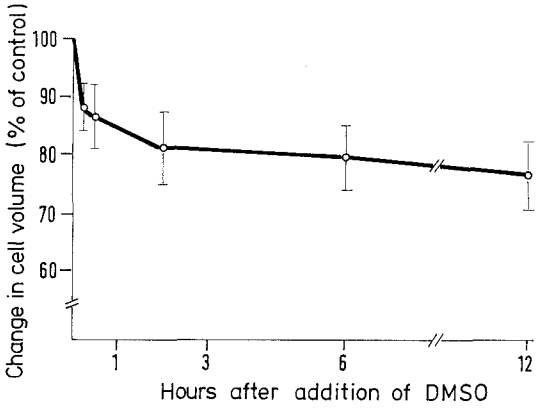

Figure 2. Changes in cell water volume of F4–6 cells after DMSO treatment. Results are expressed as percentages of the values obtained from the corresponding control cells and represent the means of three experiments in triplicate ± SE.

cytoplasm as measured by the distribution of weak acids across the cell membrane or with fluorescent pH indicators[6–11]. While the increase in intracellular pH after stimulation of cell growth is well documented in the literature, there is no information concerning the possible pH changes which would accompany the induction of differentiation when the cells reduce their proliferative character. Indirect evidence suggests that pH_i may participate in the control of the developmental program of *Dictyostelium*, and organism which has been widely used for studying the mechanism of cellular differentiation[12, 13]. Therefore, the present study was undertaken to investigate pH_i measurements in Friend erythroleukemia cells after induction of differentiation.

Murine erythroleukemia cells tranformed with Friend virus complex grow permanently in culture in vitro as proerythroblasts, but they can be induced to differentiate and synthesize hemoglobin when treated with dimethylsulfoxide (DMSO) or a variety of other inducing agents[14–16]. We have measured the change in intracellular pH in Friend erythroleukemia cells after DMSO treatment by taking advantage of the distribution properties of the weak acid 5, 5-dimethyloazolidine 2, 4 dione (DMO)[6–8, 11]. Because it is a prerequisite that cell volume be ascertained, the measurement of pH automatically provides information about the changes in cell volume after induction of differentiation with DMSO.

Materials and methods. Friend erythroleukemia cells, line F 4–6, were obtained from W. Ostertag (Heinrich-Pette-Institut for Experimental Virology, Hamburg, FRG) and have been maintained in our laboratory since 1975. The cells were grown in suspension culture in Joklik MEM+20 mM HEPES, pH 7.4, supplemented with 10% fetal calf serum. Induction of differentiation was achieved with 1.5% DMSO in logarithmically growing cells. The intracellular pH was determined by the distribution of ^{14}C-5, 5 dimethyloxazolidine 2, 4 dione (1 mCi/ml, Amersham) as described by Rottenberg[17]. Control and DMSO-treated cells were harvested by centrifugation, washed and resuspended in phosphate buffer pH 7.4 with the following composition: 115 mM NaCl; 5.5 mM KCl; 1.0 mM $MgCl_2$; 0.8 mM $CaCl_2$; 20 mM NaH_2PO_4/Na_2HPO_4; 10 mM glucose. Cell suspensions containing 4.5×10^7 cells or 3–5 mg of protein per ml were used. From this 375 µl were incubated with 375 µl of ^{14}C-DMO (1 µCi/ml) in phosphate buffer at 37 °C for 5 min. At this time the incubation period was halted when 3 samples of 200 µl were centrifuged through a 3:1 oil mixture of dibutylphthalate and dinonylphthalate (Fluka). Pellets were solubilized in 250 µl of Soluene-350 (Packard) by overnight incubation at 60 °C. Pellet and supernatant activity were then counted using Dimilume-30 (Packard) scintillation fluid.

Cell water volume was measured with ^3H_2O (Amersham; 5 mCi/ml) and corrected for extracellular water space with ^{14}C-methoxyinulin (New England Nuclear Corp.; 22.7 µCi/mg) by the method of Rottenberg[17]. This was done for each sample simultaneously with pH determination using the same method as described except that 375 µl phosphate buffer containing 1.0 µCi/ml ^{14}C-inulin and 6.0 µCi/ml ^3H_2O were used. Intracellular pH was calculated from ^{14}C-DMO distribution, cell protein[18], cell water volume and extracellular pH using the formula and pKa value given by Gillies and Deamer[19]. An average pH value of 7.55 was measured in the experiments for logarithmically growing untreated F4–6 cells.

Results. The results of the intracellular pH measurements after induction of Friend erythroleukemia cells with 1.5% DMSO are shown in figure 1. For the first 30 min following induction there is no difference in pH between control and DMSO-induced cells. At 1 h after DMSO induction intracellular pH in DMSO-treated cells began to decrease and between 3 and 9 h it was 0.16–0.18 units lower than in controls. This difference was also observed at 24 h after induction.

The cellular water volume measurements from the same experiments are given in figure 2. There was a sharp decrease in cell volume of the DMSO-treated population. As compared to controls, the induced cells show a 12% reduction in volume 15 min after DMSO addition and a 15% decrease at 30 min post-induction. There was a continuous decrease in cell water volume of the DMSO-treated cells, reaching a volume at 12 h which was 23% reduced.

Discussion. By the DMO distribution method an intracellular pH value of 7.55 was measured in logarithmically growing F4–6 cells. Similar pH values have been measured in transformed murine lymphocyte cell lines during the exponential growth phase using this method[5]. After DMSO-treatment there was a decrease in pH. The maximum change of 0.18 pH units occurred at 6–9 h. This decrease in pH corresponds reversely to the increase in pH which has been reported after stimulation of proliferation with mitogens in other cell lines[7–10]. This shows that, while on the one hand, stimulation with mitogens leads to cytoplasmic alkalinization, on the other hand, the induction of differentiation of Friend erythroleukemia cells with DMSO, whereby the cells lose their reproductive potential, leads to a decrease in intracellular pH. Our results support the hypothesis that intracellular pH plays an important role in the regulation of cell growth.

The increase in intracellular pH during mitogen stimulation in fibroblasts results from the activation of the Na^+/H^+ exchange system[8–11]. Since DMSO-induction of Friend-erythroleukemia cells results in a decrease in Na^+ transport into the cell[20,21], it is possible that reduction in the Na^+/H^+ exchange system is responsible for the decrease in pH after DMSO treatment. This will require further investigation, since the activity of the Na^+/H^+ exchange system is relatively low during normal growth conditions in the exponential phase[22].

Our results also show a brisk decrease in cell water volume after DMSO treatment of F4–6 cells. This occurs within 15 min. With other techniques the decrease in cell volume after DMSO induction was first observed after 8–10 h[23,24]. The quick volume decrease may be indicative of changes in the cell membrane during the very early phase of the induction of differentiation with DMSO.

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Inhibition of sensitized leukocyte's in vitro reactivity by circulating immune complexes in prostate cancer

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Summary. Circulating immune complexes in the sera of patients with confirmed histological diagnosis of carcinoma of the prostate, were found to interfere in the sensitized leukocyte's in vitro reactivity to prostate cancer associated antigen as evaluated by tube leukocyte adherence inhibition assay, thereby suggesting an inhibitory role of such serum factors in host's anti tumor cell mediated immune responses.
Key words. Circulating immune complexes; prostate cancer, inhibition.

The failure of immune responses in tumor bearing hosts to control tumor growth results from the intervention of a series of host factors that diminish the effectiveness of cell mediated immunity. One of such factors are antigen/antibody complexes which are reported to have an adverse effect on cell mediated reactions in humans as well as in experimental tumor systems. In a number of studies circulating immune complexes (CIC) were found either to reduce[1] or abrogate [2–5] the cell mediated cytotoxicity against cultured tumor cells and even interact with the receptors on cells resulting in the release of biologically active mediators which may interfere with cellular functions or the cells' recognition mechanism[6]. The presence of such complexes in patients with cancer of the prostate (CaP), and the alteration of such levels by anti neoplastic therapy have already been reported[7,8]. The present investigations were undertaken to evaluate any in vitro inhibitory effect of prostate cancer CIC on cells' recognition mechanism.

Materials and method. Patients. Blood samples were obtained from 10 patients with confirmed histological diagnosis of adenocarcinoma of the prostate who were receiving various types of therapy at the time of evaluation. Serum samples were stored at −20 °C. In order to evaluate the effect of CIC on cellular functions, leukocytes from 5 control subjects with no clinical signs or symptoms of any malignancy or immunologic disorder were used.

Polyethylene glycol precipitation assay for measuring CIC. Serum circulating immune complexes in the sera of patients with cancer of the prostate were measured as detailed previously[7]. Briefly, diluted serum samples were mixed with a 3.75% solution of polyethylene glycol (MW 6000), and with 0.1 M borate buffer to serve as controls. All tubes were run in