from those previously published[8–10] in two ways: 1. antiserum in varying concentrations is first titered against constant amounts of radioantigen and antiglobulin antibody and 2. a second dimension is provided by titrating varying dilutions of the radioantigen against constant amounts of the homologous antiserum determined in the first step. The combining of steps 1 and 2 (Figure 1) at each precipitation point along the serum dilution axis results in 2-dimensional prospective, showing the multiple antigenic determinants which have contributed to anti-HCG antibody formation. The individual precipitin curve for each antigen in the system can then be constructed (Figure 3) from the second dimension.

Radio-coprecipitation (2-DRCP), as performed in double dimension, will allow the investigator to achieve optimal serum dilution analysis to detect specific antigenic constituents in the nano- and pikogram range (5 ng in the present study). The present method of 2-DRCP may be applied to screening sera for radioimmunoassays to determine points of optimal precipitation. This new technique, in turn, may relate the purity of the antigenic preparations employed in radioimmunoassays.

Zusammenfassung. Neue Variante des Radio-Immundiffusionstests mit Antigenanalyse von menschlichem Gonadotrophin.

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The Influence of Proteolytic Enzymes Inhibitor on the Course of Lymphocyte Transformation in vitro

It is known that both phytohemagglutinin (PHA), streptolysin S, antilymphocyte sera, antigen and antigen-antibody complexes induce lymphocytes transformation in vitro[1–5]. Transformation stimulators enhance oxygen consumption[6], RNA synthesis[7], and also cause changes in the morphological character of lymphocytes. Using histochemical methods, HIRSCHHORN et al.[3] demonstrated that in the course of the transformation the content of acid hydrolases in the stimulated lymphocytes increases. Moreover, the PHA simplifies the destructive influence of streptokinase S on lymphocyte lysosomes[8]. DIENGDOH and TURK[9] were able to demonstrate that lymph node cells contain more acid hydrolases after antigen stimulation. Analogical changes were observed in blood leukocytes of rabbits after allogenic skin transplantation[10].

The present communication is to show the role of proteolytic enzymes inhibitor in the course of lymphocyte transformation in vitro.

Material and methods. The investigations were carried out on lymphocytes obtained from the spleen or lymph nodes of immunized guinea-pigs weighing 350–450 g. Male guinea-pigs were immunized by human serum albumin (HSA) produced by Biomed, Poland. Antigen was injected with mineral oil as an adjuvant, in the proportion 1:1, to the whole quantity of 2 mg HSA for 1 guinea-pig. The experiments were carried out 20 to 30 days after the immunization. Cells of the spleen and lymph nodes were prepared in suspensions containing $2–4 \times 10^6$ lymphocytes/ml. The lymphocyte cultures were incubated in tubes at 37 °C for

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Influence of inhibitor of proteolytic enzymes on transformation rate, oxygen consumption and incorporation of ^{14}C-glycine by lymphocytes immunized guinea-pigs in vitro

	Control	Inhibitor	PHA	PHA + inhibitor	Antigen	Antigen + inhibitor
Number of lymphoblasts/500 spleen lymphocytes	40 ± 26.6	30 ± 15	200 ± 67.7	30 ± 21.2	262 ± 95	19 ± 5.6
Number of lymphoblasts/500 lymph node lymphocytes	60 ± 30	36 ± 25	190 ± 75	13 ± 8.0	252 ± 100	18 ± 7.1
Oxygen consumption in μl/10^6 cells	1.49 ± 0.34	1.31 ± 0.1	1.74 ± 0.25	1.78 ± 0.1	1.81 ± 0.3	1.8 ± 0.2
^{14}C-glycine incorporation (impulses/mg/min)	1544 ± 21	1516 ± 61	1579.3 ± 73	1516 ± 61	2302.5 ± 120	2262.5 ± 81

72 h, in tissue culture medium 199 (Biomed, Poland) with addition of 10% calf serum. The tubes were rotated and kept in an air atmosphere with addition of 5% CO_2. Parallel investigations were made in the following experimental groups: 1. lymphocytes, 2. lymphocytes + PHA, 3. lymphocytes + PHA + inhibitor, 4. lymphocytes + antigen, 5. lymphocytes + antigen + inhibitor. These experiments were performed using PHA P (Difco) 0,2 µl/ml, antigen 2 µg/ml inhibitor (Zymofren Specia) 200 units/ml. After the appropriate period of cultivation, smears were made and stained with Giemsa dye. The cells were evaluated under the light microscope at 1000 magnification. The defined number of lymphoblasts was established for 500 cells in the smears. The percentage of survival was estimated under neutral red[11]. The toxic concentration of inhibitor was estimated according to the method of MERCHANT et al.[12]. This concentration after a 48 h cultivation period caused a depression in cell growth around the cultivated fragments of spleen. Inhibitor in a final concentration more than a half of this toxic concentration was added to the lymphocyte culture. Oxygen consumption was measured to the routine method in a Warburg apparatus[13]. In each Warburg tube, lymphocyte suspension was adjusted to a concentration 4×10^6 per 3 ml. Protein synthesis was measured by means of an incorporation of ^{14}C-glycine (Radiochemical Centre, Amersham, Buckinghamshire, England). 2 µCi of ^{14}C-glycine was added 18 h before the end of the experiment. After 72 h cultivation, the lymphocytes were washed 3 times with 0.85% NaCl and cell protein was precipitated with ice-cold 5% trichloracetic acid (TCA). The number of impulses was measured in a Packard liquid automatic scintillation counter and calculated for 1 mg TCA precipitated cell protein.

Results. Toxic concentration of inhibitor equalled 500 µg/ml. To the lymphocyte culture 200 µg/ml of this inhibitor was used. The lymphocyte survival after 72 h of culture equalled 80 ± 4%. The cell viability did not differ significantly in the proposed experimental groups. The results obtained from 6 experiments are presented in the Table.

The inhibitor of proteolytic enzymes decreases significantly the transformation rate of lymph node and spleen lymphocytes in vitro, either after PHA or antigen stimulation and lowers the ^{14}C-glycine incorporation into cell protein, but does not alter the oxygen consumption.

Discussion. We have demonstrated an inhibition of antigen or PHA induced lymphocyte transformation in vitro by the inhibitor of proteolytic enzymes. Therefore a number of obvious causes have to be considered before interpreting the significance of inhibition. The inhibition of transformation might simply reflect a destroying of the responding cells. In our investigation we have found no gross signs of toxicity as reflected by alternation in cell viability.

The inhibitor of transformation may influence the protein synthesis. WEISSMANN et al.[14] suggest that proteolytic enzymes abolish the suppressing action on the cell protein synthesis. For this reason, the inhibitor might be acting as a repressor of protein synthesis and affect lymphocyte ability to respond to PHA or antigen. The observed fall of ^{14}C-glycine incorporation after inhibitor administration in either stimulated or nonstimulated lymphocytes confirms the hypothesis of the role of proteolytic enzymes in cell protein synthesis. The subtle impairment of normal cell function, although not resulting of the cell in death, did interfere with the cellular ability to respond to the stimuli. According to this suggestion, the inhibitor of the proteolytic enzymes decreasing cell protein synthesis blocks the lymphocyte transformation in vitro. The above-mentioned phenomenon suggests that the protease plays an important role in blastic transformation of immunological immature cells.

Résumé. Nous avons observé que l'inhibiteur d'enzymes protéolytiques (Zymofren-Specia) inhibe la transformation blastique des lymphocytes sensibilisés de cobaye évoquee par la phytohémagglutinine ou l'antigène. Nous n'avons pas observé d'influence ce de produit sur la consommation d'oxygène.

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Antigenicity of *Poecilia* Sperm

In viviparous teleost fish of the family Poeciliidae, sperm are stored for periods of up to 10 months within the ovary of the female[1]. A number of successive broods may thus result from a single insemination. The storage of sperm within the body of the female, and in intimate association with the tissues of the ovary, presents a number of physiological problems. We have investigated the immunological aspect of this situation: how genetically foreign sperm cells can survive within an immunologically hostile environment.

Several explanations are possible in principle; the ovary, perhaps by virtue of its secretion of hormones such as oestradiol, known to impede allograft rejection in certain circumstances[2], might be a favourable site for allograft survival; the sites of sperm storage might lack lymphatic drainage and thus constitute 'immunologically privileged' sites[3]; or the sperm themselves might be antigenically null. Tissue allografts within the ovary are destroyed as rapidly as those to other parts of the body[4], hence the first hypothesis must be rejected. We have investigated the antigenicity of the sperm of a poeciliid fish, *Poecilia reticulata* Peters, the Guppy.

Ejaculates of sperm were obtained from male *Poecilia* anaesthetized in a 1:4,000 solution of Sandoz MS 222[5] by manual erection of the gonopodium (intromittent organ) and gentle pressure on the abdomen in the region of the

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