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 $4 \times 10^6$ 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.

H. TCHÓRZEWSKI and A. DENYS

*Department of General and Experimental Pathology, Pl. 9-go, Maja 1, Lódź (Poland), 16 July 1971.*

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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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testis. The semen was collected in 0.6% saline in the nozzle of a fine glass pipette and dispersed in approximately 0.1 ml of Freund's Complete Adjuvant (Difco preparation).

Ten adult *Poecilia* (5 ♂♂, 5 ♀♀) were then given i.p. injections, under anaesthetic, of 0.05 ml of the sperm suspension (containing approximately $2 \times 10^5$ sperm), and matched controls were injected with the same volume of adjuvant as a control. After recovery from the anaesthetic, the recipients were returned to holding tanks.

Teleosts do not typically produce high titres of humoral antibody, and as the volume of serum available for assay was small (no more than 0.08 ml from a fish 25 mm in length), the presence of antibody was detected by its immobilizing effect on motile sperm. Suspensions of ejaculated sperm were maintained in a hanging drop culture in Ringer's solution. Poeciliid sperm maintained in this way retain their motility, in aerobic conditions, for several hours[6]. The effects of blood obtained from the experimental fish on the motility of the cultured sperm was assessed.

Fourteen days after injection, the experimental fish were killed and approximately 0.05 ml of the blood of each added to sperm suspensions. The sperm suspensions were then observed at 15 min intervals until all motility had ceased.

The results are given in the Table. Sperm to which blood from sperm-injected fish had been added ceased movement in a significantly shorter time ($p < 0.001$) than those to which the added blood came from control fish. We attribute this to the induction, in the recipients of sperm injections, of sperm-immobilizing antibody.

Time (h) by which all motility had ceased in sperm cultures to which blood had been added from sperm-injected and from control fish. Means of 5 experimental and control males and female fish quoted ± S.E. Between treatments $F_{10}^1 = 22.2$, $p < 0.001$.

| | Sperm in adjuvant | Adjuvant |
|---|---|---|
| Males | 1.45±0.22 | 2.65±0.22 |
| Females | 1.30±0.37 | 2.60±0.23 |
| combined | 1.38±0.20 | 2.63±0.15 |

The sperm of *Poecilia* are, therefore, antigenic. As the sperm used for the injection came from different males from those which supplied sperm for the assay, it is possible that the antigens in question are sperm – rather than allospecific. Sperm stored in the ovary do not, therefore, avoid immune rejection by being antigenically null.

Histological examination of the ovaries of the female recipients of sperm injections showed no appreciable decrease in the numbers of sperm stored, compared with the control fish. It seems, therefore, probable that the induction of antibody to sperm in the female by i.p. injection has no effect on sperm stored within the ovary. Stored sperm do not, therefore, evade allograft rejection by the alymphatic nature of their storage site.

*Résumé.* On a retenu des spermatozoïdes pendant quelques mois dans l'ovaire des femelles du poisson téléostéen vivipare *Poecilia*. L'aspect immunologique de cette situation a été étudié pour savoir si les spermatozoïdes sont antigéniques. Le sérum des poissons traités par des injections de spermatozoïdes en suspension immobilise les spermatozoïdes actifs dans un temps moyen de moins de 2 h, ce que ne fait pas le sérum des poissons témoins. On peut conclure que la formation d'anticorps immobilisants a été éliminée et que les spermatozoïdes sont en effet antigéniques.

P. J. HOGARTH and CARMELINA M. SURSHAM

*University of York, Biology Department, Heslington, York Y01 5DD (England),*
*21 October 1971.*

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## Immunofluorescence of Arginine Deprived Cells Infected With Adenovirus Type 12

The importance of arginine for the synthesis of many DNA animal viruses is well recognized[1-4]. Adenovirus replication is especially sensitive to arginine deficiency requiring this amino acid for assembly and maturation of virions[1,5]. Arginine-rich proteins have been isolated from disrupted viral particles[6,7]. It was demonstrated recently that L-canavanine, a competitive inhibitor of arginine[8], incorporated into virus proteins could reduce viral yields[9]. Studies by immunofluorescence have shown that arginine deficiency prevents the synthesis of the late 'p' antigen of adenovirus type 5[5,10]. SHIMOJO et al.[11] reported a similar antigen which appeared as large nuclear fluorescent dots (FD), which were synthesized in human cells infected with adenovirus type 12. This antigen can be demonstrated by indirect immunofluorescence when these cells are reacted with sera from certain hamsters bearing tumors induced by adenovirus type 12 (Figure 1). The purpose of this report is to demonstrate that synthesis of this antigen was sensitive to arginine deprivation and that it is probably identical with the arginine-rich internal viral protein.

*Material and methods.* Methods used to show the relationship of arginine to this antigen include the growth of KB cells infected with adenovirus type 12 on arginine deficient media as well as the addition of L-canavanine, a competitive inhibitor of arginine, to the medium. The growth medium consisted of Eagle's MEM with and without arginine supplemented with 10% fetal calf serum (FCS). The maintenance medium was the same but contained 2% FCS. Cells were grown on coverslips in Leighton tubes. Methods of handling Leighton tube cultures and methods of indirect immunofluorescence have been described previously[12].

*Results and discussion.* Cells maintained on either arginine-free media or 100–200 µg of L-canavanine/ml and examined by indirect immunofluorescence showed a slight reduction in intensity of 'T' antigen. The latter was identi-

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