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 2×10^5 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.

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[6] P. J. HOGARTH, unpublished results.

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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fied as fluorescent flecks (FF). A slight reduction of viral antigen was also noted in these cells when they were reacted with antiviral sera prepared in rabbits. The lack of arginine, however, had a marked effect on the presence of the FD antigen. With increasing concentrations of L-canavanine, the number of cells with demonstrable FD antigen was reduced. A concentration of 100 µg/ml in the growth medium, added after virus absorption, was sufficient to allow only 2 or 3 cells per coverslip to develop the specific intranuclear fluorescence. Concentrations of 125 to 150 µg/ml of L-canavanine in the medium completely inhibited this fluorescence. When the cells were fed with arginine-deficient or L-canavanine-containing media prior to virus absorption, lower concentrations of L-canavanine were effective in inhibiting the synthesis of this antigen.

Since L-canavanine is a competitive inhibitor of arginine, experiments were devised to show that excess arginine could reverse the inhibitory effects of L-canavanine. When 2X arginine (205 µg/ml) was used in the maintenance medium, the inhibitory effects of 100 to 200 µg L-canavanine, as determined by indirect immunofluorescence, were reversed and FD antigen was found in the infected cells. When infected cells were fed with arginine-deficient or L-canavanine-containing media following virus absorption, incubated for 24 h, and then returned to complete media containing 2X arginine, a very rapid appearance of fluorescence identifiable as FD was noted. Within 3 h the typical FD began to appear in the nucleus of cells maintained on the arginine-deficient media but somewhat longer was required for the cells maintained on L-canavanine.

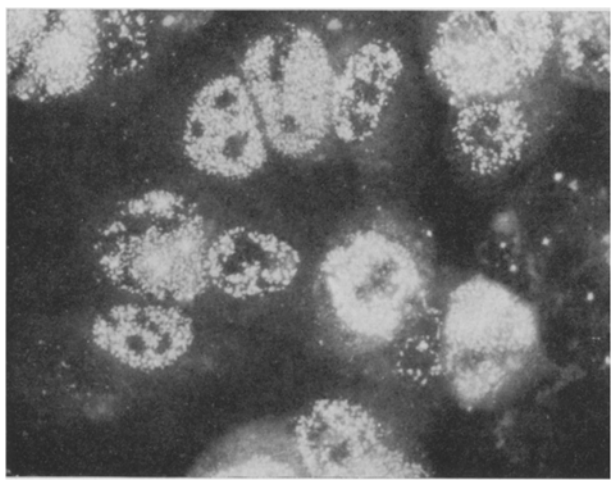

Fig. 1. Fluorescent dots in KB cells infected by adenovirus type 12

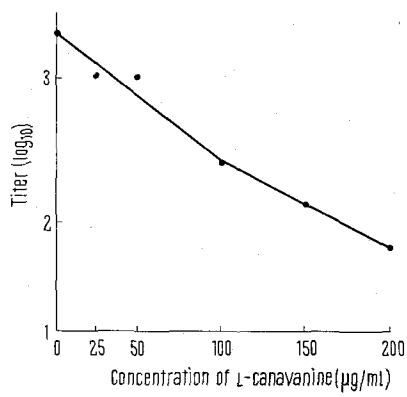

Fig. 2. Effect of L-canavanine on adenovirus 12 titer. Infected cells were incubated 30 h, lysed, then incubated with HEK cells in microtiter plates and read after 10 days.

Studies were initiated to show what effects L-canavanine would have on viral infectivity. Replicate KB tube cultures were infected at high multiplicities (10^3 $TCID_{50}$), washed to remove unabsorbed virus, and fed with media containing various concentrations of L-canavanine. After 30 h, these cultures were washed, pooled, frozen and thawed 5 times, diluted, and mixed with human embryonic kidney (HEK) cells and dropped into microtiter plates. Results following 10 days of incubation can be seen in Figure 2. There was approximately 30 times as much virus produced in the complete medium as compared with the cells in the growth medium containing 100 µg/ml of L-canavanine. Similar results were obtained using arginine-free media.

These results suggest that the 'FD' antigen[11,12] was sensitive to arginine deprivation, which is known to prevent virus maturation. Since this protein was not observed in cells grown in arginine-deficient or L-canavanine-containing media, it seems reasonable to conclude that the internal adenovirus protein may be identical with the intranuclear protein described by these investigators. The limits of the immunofluorescent technique do not preclude the possibility that this antigen was synthesized in amounts not detectible by the above method. It is likely that lesser amounts were synthesized through the arginine pool present in the cells, as well as that contained in the media, but not in sufficient quantities to develop the characteristic fluorescent pattern.

L-canavanine toxicity to cells was an important problem in the experimental design. Concentrations of 1 µg/ml were sufficient to cause cytopathology if maintained on the cells for extended periods of time. When cells were maintained on L-canavanine following viral infection, more time was required for these cells to synthesize visible amounts of FD antigen than those on arginine-minus media following replacement with arginine-rich media. This may indicate the necessity for arginine replacement of the L-canavanine which is incorporated into viral proteins[9].

Results by WINTERS and RUSSELL[13] indicated that extracts from HeLa cells growing in complete media and infected with adenoviruses may, upon addition to infected cells maintained on arginine-deficient media, allow the assembly of virions. This suggests the possibility that viral cores could be substituted between adenovirus types[14] and confirms that the synthesis of an arginine-requiring viral protein is necessary for viral maturation[1].

Zusammenfassung. KB-Zellen, die mit Adenovirus 12 infiziert waren und entweder in argininfreiem Medium oder in Medium, das 125 µg/ml L-Canavanin enthielt, gehalten wurden, entwickelten kein nukleares Antigen, das durch indirekte Immunofluoreszenz ermittelt werden konnte.

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[15] We thank Mrs. REBECCA CASTLEBERRY for her excellent technical assistance.