Table 1 Protection by antiserum of newborn mice against HSV infection

Expt	Mouse strain (Age, d)	HSV type (strain)	Challenge dose (LD_{50})	Dose of antibody (U per mouse)	Dead mice/ Total mice	% Mortality	% Protection	Combined% protection*
1	CDF-1 (1)	1 (VR3)	10	0	7/7	100		
			10	12,000	1/8	13	87	
2	Swiss (1)	1 (VR3)	2	0	6/9	67		
				12,000	0/10	0	100	
3	Swiss (1)	1 (VR3)	20	0	19/21	90		
				12,000	1/19	5	94	94†
4	CDF-1 (1)	1	100	0	6/7	86		
			100	12,000	6/7	86	0	0
5	BALB/c (1)	2 (MS)	2	0	3/8	38		
				600	5/5	100	0	
			20	0	7/7	100		
				600	7/7	100	0	
6	Swiss (1)	2 (MS)	20	0	18/22	82		
				600	9/22	41	50	18
7	Swiss (4)	2 (MS)	1	0	3/5	60		
				12,000	0/6	0	100	
		2 (MS)	3	0	5/5	100		
				12,000	4/8	50	50	
8	Swiss (2)	2 (MS)	2	0‡	3/5	60		
				12,000	2/8	25	58	
9	Swiss (1)	2 (MS)	3	0	5/5	100		
				12,000	2/6	33	67	
10	Swiss (1–2)	2 (MS)	3	0‡	7/8	88		
				12,000	2/14	14	84	71†
11	Swiss (1–3)	2 (MS)	300	0	18/18	100	0	
			300	12,000	25/25	100	0	0

* Average % protection for preceding experimental group.
† $P < 0.005$.
‡ Normal rabbit serum.

of newborn or young animals infected intranasally[13,19], intradermally[13], intracerebrally[14,15,17], intraperitoneally[14], intravaginally[18] or subcutaneously[16]. Because of these earlier findings, human immune globulin treatment in man had been considered[1,12,19,24]. If proper conditions can be defined to protect newborn infants with hyperimmune human globulin, this treatment would offer several advantages over present methods: for example, administration of human immune globulin to newborns is much safer than surgical delivery of the babies or than use of toxic drugs. Finally, combination of protective antibody with other antiviral agents, such as interferon, might enhance protection as with vaccinia virus infection of mice[23].

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Type C virus in lymphosarcoma in northern pike *(Esox lucius)*

THE northern pike (*Esox lucius*) is a highly prized freshwater fish, both as a game fish and as a commerical species. Epizootics of lymphosarcoma occur widely in North American pike[1] and in the Old World[2,3]. The tumour in pike has been found with an overall frequency of 20.9%, which is the highest frequency of a malignant neoplasm in any known free living vertebrate[1]. All pike with the tumour have cutaneous lesions, and epizootiological evidence suggests that the disease is transmitted horizontally by contact during spawning[1]. The tumour is transplantable and evidence of cell-free transmission has been found[1,4]. In an attempt to resolve the aetiology of the disease, we have investigated the presence of oncornavirus in pike lymphoma. Since all known RNA tumour viruses possess the enzyme reverse transcriptase, we have sought the activity of this enzyme in post-mitochondrial particulate fractions prepared from pike lymphoma tissue.

Pike lymphomas were analysed as described in the legend

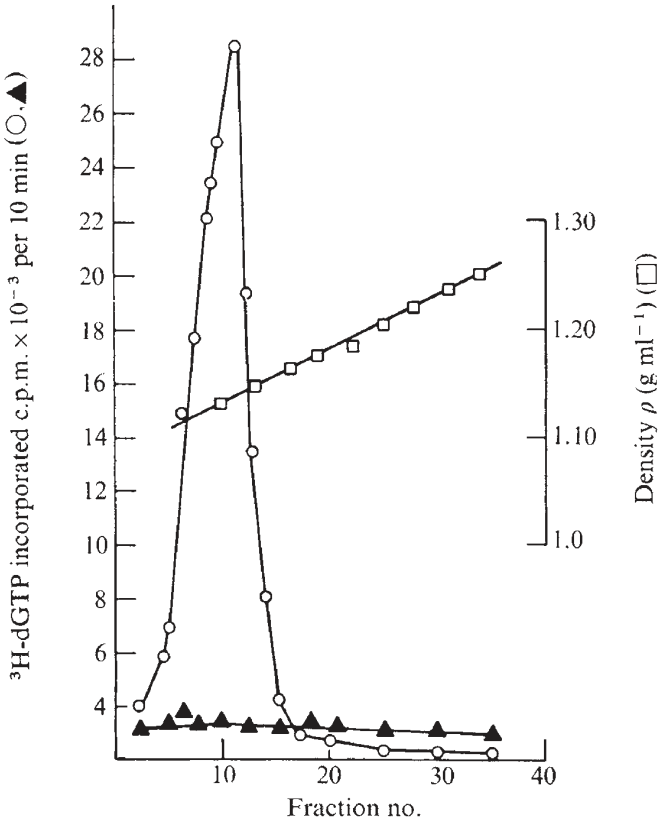


Fig. 1 Identification of the density of reverse transcriptase in a postmitochondrial extract of pike lymphoma by sucrose gradient centrifugation. Frozen pike lymphoma tissue (10 g) was homogenised at 4 °C in 2 volumes of 0.05 M Tris-HCl (*p*H 7.5), 0.01 M dithiothreitol and 0.25 M sucrose. It was then sonicated for 1 min and the suspension was centrifuged at 1,000*g* for 30 min at 4 °C. The supernatant was centrifuged at 16,000*g* for 30 min. The supernatant was again centrifuged at 100,000*g* for 1 h at 4 °C in an SW27 rotor. The resulting pellet (P-100) was suspended in 0.5 ml of 0.01 M Tris-HCl (*p*H 7.5) containing 0.01 M dithiothreitol and layered in a 13 ml 25–60% (w/v) sucrose gradient in TNE and centrifuged at 100,000*g* for 22 h in an SW41 rotor. Approximately 40 fractions were collected from the top by Buchner densiflow. A sample (20 µl) was added in a reaction mixture of (100 µl) containing 100 mM Tris-HCl (*p*H 8.3), 50 mM KCl, 1 mM $MnCl_2$, 4 mM dithiothreitol for poly(Cm).oligo(dG) (20 µg ml^{-1}) and 0.125 M ^{3}H-dGTP. Reaction mixtures were incubated at 20 °C for 1 h and stopped by addition of 10% trichloroacetic acid (TCA). Acid precipitable material was collected on Whatman GF/C filters and the radioactivity was counted. Normal pike tissue was processed in identical conditions and assayed as described above. ○, Pike lymphoma; ▲, normal pike.

to Fig. 1. A cytoplasmic particulate fraction was isolated from the tissue. After further fractionation on sucrose gradients, fractions were assayed for the presence of reverse transcriptase able to utilise poly(Cm)·oligo(dG), a template primer specific for viral polymerases[5]. Reverse transcriptase activity was associated principally with the particulate fraction sedimenting at a density of 1.16 g cm^{-3}. Parallel gradients run with purified Rauscher leukaemia virus were fractionated and assayed similarly for reverse transcriptase activity. Peak activities also occurred at a density of 1.16 g cm^{-3}. When normal pike tissue was analysed identically, no reverse transcriptase activity was detected in any gradient fraction. Thus the reverse transcriptase activity was unique to the lymphoma, and was associated with a particulate fraction corresponding to the density of known RNA tumour viruses.

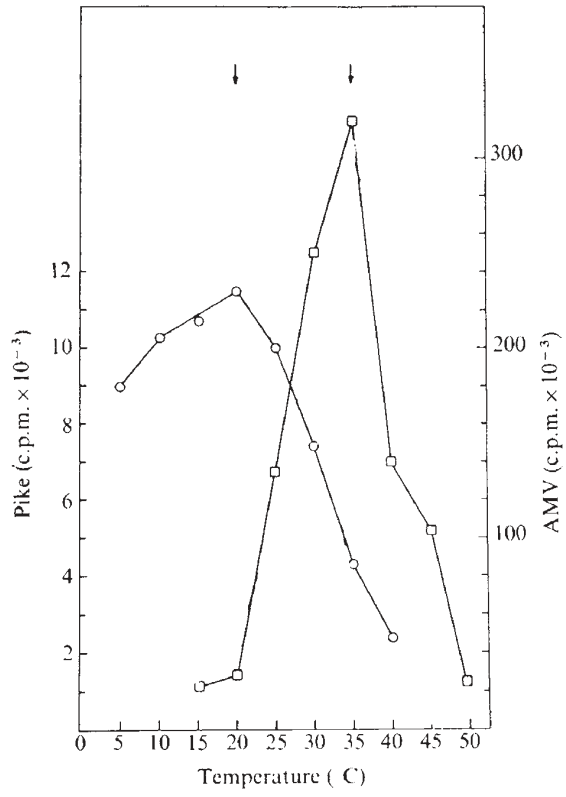
The seasonal nature of the appearance of the pike lymphoma prompted us to examine the temperature profile of the reverse transcriptase activity. As Fig. 2 shows, the enzyme activity from pike lymphoma exhibited 82% of its maximum activity at 5 °C, with an optimum at 20 °C while only 35% was retained at 35 °C. In contrast, reverse transcriptase purified from avian myeloblastosis virus (AMV) has a temperature optimum of 35 °C with only 0.6% of optimal activity at 20 °C and no detectable activity at 5 °C.

Fractions between 1.15 and 1.17 g cm^{-3} were diluted to less than 10% sucrose, pelleted and processed for electron microscopy. Figure 3 shows the appearance of the numerous type C virus-like particles present in these gradient fractions. Most particles appear similar to typical type C virus particles of avian and mammalian origins, but a more careful categorisation must await the detection of budding and immature forms of the virus.

The results reported here demonstrate that pike lymphoma contains poly(Cm)·oligo(dG)-directed DNA polymerase activity present in a structure with the density of an RNA tumour virus. These particles are not disrupted by physical manipulation and maintain their characteristic density when centrifuged repeatedly. This is the first report of the presence of reverse transcriptase in a fish, which is phylogenetically the most primitive animal in which the enzyme has been detected.

The aetiological significance of the type C virus-like particles and its associated reverse transcriptase is unknown. The disease in nature exhibits marked seasonal periodicity, with tumours developing during the cold water periods of fall and winter[1], when mean water temperatures are 12 and 4 °C, respectively. Field epizootiological studies have revealed that the tumours frequently regress spontaneously during the summer months[1], with typical water temperatures varying from 21 to 30 °C. The high activity of the enzyme at low temperatures correlates with the water tem-

Fig. 2 Temperature profiles of partially purified pike and purified AMV reverse transcriptase. Samples were incubated for 30 min in the corresponding reaction mixtures at the temperatures indicated. Reaction mixture temperatures were accurately controlled by using a Lauda circulating water bath. Reactions were stopped by addition of cold 10% TCA, and amounts of ^{3}H-dGMP incorporated into acid-insoluble product was determined. ○, Pike; □, AMV.



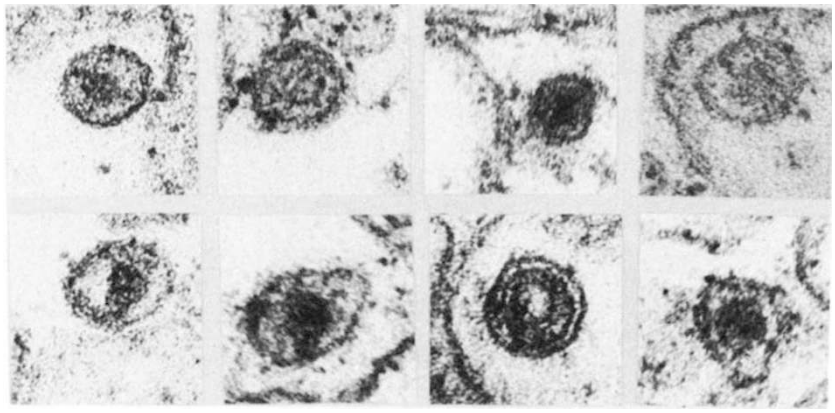


Fig. 3 After frozen tumour had been thawed, homogenised, and fractionated on a sucrose gradient, fractions with densities between 1.5 and 1/17 g cm^{-3} were pooled, diluted and pelleted at 100,000*g*. The pellet was fixed for 2 h in 3% glutaraldehyde in Milling's buffer, washed for 30 s in buffer, post-fixed for 1 h in 1% osmium tetroxide, washed for 2 h in distilled water, stained *en bloc* with 2% uranyl acetate in 50% ethanol, dehydrated in ethanol and propylene acids, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and examined at 80 kV in a Siemens 101 electron microscope (×160,000).

peratures at which the tumour develops in nature. The warm water period of summer (non-permissive temperatures) may explain the seasonal periodicity of the disease and the mechanism of the spontaneous regressions observed. The disease in fish offers a unique probe, facilitating both *in vivo* and *in vitro* assays to resolve 'turn on' and control mechanisms of oncornavirus-induced transformation, as both cell cultures and hosts can be placed at permissive and non-permissive temperatures.

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Virulence of different strains of *Toxoplasma gondii* and host response in mice

THE protozoan *Toxoplasma gondii* has a world-wide distribution. It is an obligate intracellular parasite and appears in many species of mammals and birds. Animals that survive the acute stages of *Toxoplasma* infection probably remain infected for life[1]. The RH strain of *Toxoplasma gondii*, originally isolated by Sabin[2] and widely used in the routine Dye test[3] is extremely pathogenic to mice, LD_{100} by intraperitoneal infection being below 10 tachyzoites[4]. Moreover, it is difficult to obtain survival after RH challenge of mice immunised with a strain of lower virulence[5]. Such survival without the use of chemotherapy has, however, been reported[1,6,7]. The RH strain was not totally eradicated[7] from immunised mice even 7 weeks after challenge. How do immunised mice suppress the RH strain—by total elimination or making it possible for the RH strain to remain in the body as does the immunising strain? Furthermore, in the latter case, would the inherent virulence of the RH strain become changed? We here report that *Toxoplasma* possessing the full virulence of the original RH strain, could be isolated from mouse brains 14–18 months after RH challenge of mice which had first been immunised with the Beverley strain. This applied, however, to a proportion of the mice; in the brains of the other mice only the immunising strain (Beverley) was found.

Brain suspensions from test groups (mice infected with the Beverley strain and reinfected with the RH strain) were injected subcutaneously into normal mice (Fig. 1). In two of the test groups (1 and 4) all mice died as did the RH controls. In the others a varying proportion of the mice succumbed, an event which also occurred in Beverley infected controls. In this comparison the RH control infection started from the tachyzoite stage, whereas the others started from a cystic stage. To establish a comparison with all infections arising from the tachyzoite stage, the following procedure was undertaken : Two mice from each of the test groups 1, 2 and 3 as well as the RH and Beverley controls were killed after 6–9 d, brain suspensions were made and injected into groups of normal mice (Fig. 2). In test groups 2 and 3 all mice survived as did the Beverley controls. The surviving mice had become Dye test-positive, proving that they had gone through a *Toxoplasma* infection. In test group 1 all mice died, the survival time being almost identical to that found for RH-infected mice, indi-

Fig. 1 Survival rates of mice after infection with *Toxoplasma gondii*. Brain suspensions of mice (female NMRI/Bom) which had been infected with *Toxoplasma gondii* were injected into normal mice. Brains were derived from mice which had been infected in three different ways. ●, Test groups. Mice which had been infected first with the Beverley strain and then with the RH strain ($5^1 \times 10^2$ or 5×10^5 tachyzoites intraperitoneally) 2 weeks–4 months later. 14–18 months after the RH challenge the brains were dissected out. Number of the individual test group as shown. △, Mice which had been infected merely with the Beverley strain 12 months previously. ○, Mice with a RH infection of 4 d duration. Brains were mortared in 0.9% phosphate-buffered saline. Each brain suspension to be tested was prepared from one mouse or from two parallel mice. The brain suspensions were diluted so that one brain would provide enough material for infection of 20 new mice. Aliquots (0.3 ml) of the brain suspensions were injected subcutaneously to each normal mouse (9–20 mice in each group).