Cell-Free Transmission of Lymphosarcoma in the Northern Pike *Esox lucius* L. (Pisces; Esocidae)

The occurrence of lymphosarcoma in the northern pike, *Esox lucius* L., in Ireland, and the histology of the tumours, have been described[1]. Tumours are sited in many organs in the body but most often in the jaws. Viral aetiology has been suggested[2]. Electron-microscopic examinations of some of the tumours from Irish pike have not revealed virus particles[3,4]. However, 2-stage serial transmission of the lymphosarcoma has now been achieved, using cell-free homogenates of tumour tissue, which suggests that a virus or mycoplasma may in fact be involved.

Tissue was taken immediately after death from a jaw tumour of a 2 kg female pike with lymphosarcoma (C61). The tissue was homogenized 1:1 with Dulbecco phosphate-buffered saline (Oxoid), filtered through a 0.22 μ Millipore and injected i.p. into 2 healthy adult pike, one male (1P1) and one female (1P2). 1P1 died after 55 days; macroscopically it was normal, but a detailed examination showed that the normal structural organization of one thymus gland was disrupted[5], though other tissues examined (jaw, liver, spleen, anterior, mid- and hind-kidney) were normal. 1P2 developed obvious tumours after 178 days; it was killed after 208 days, at which stage tumours had developed in the upper jaw, the right nasal cavity, and the dorsal body wall; and all organs examined histologically showed neoplastic involvement (lower jaw, thymus, liver, spleen, anterior, mid- and posterior kidney).

Homogenates of the jaw tumour and kidney of 1P2 were prepared as before immediately after death, and injected i.p. into 4 adult healthy male pike (1P4–1P7). 2 of these fish died, one after 48 days, one after 55 days; the remaining 2 were killed, as they were moribund, after 55 days. These 4 injected pike did not show macroscopic tumours, but histological examination of the thymus glands, upper and lower jaws, liver, spleen, anterior mid- and posterior kidney, skin and dorsal muscle, revealed in each organ, in each fish, neoplastic masses and extensive infiltration and tissue replacement, similar in appearance to spontaneous lymphosarcoma.

In 3 separate experiments, control injections were carried out. 4 healthy adult pike, 3 male and 1 female, were injected i.p. with cell-free homogenates, prepared as before, but of tissue from 3 healthy pike. Control I died after 21 days; Control II was killed after 55 days; and Controls III and IV were killed after 144 days. Neither macroscopic nor microscopic changes, neoplastic or otherwise, were detected in any of these control pike, though in each case, detailed histological examination was made of upper and lower jaws, thymus glands, liver, 3 regions of the kidney, spleen and dorsal body wall.

The results of these experiments suggest that a virus, or organism of comparable size, may be the aetiological agent of the pike lymphosarcoma, since neoplastic change was produced in healthy pike by injection of tumour tissue but not of normal tissue, even though the homogenized tissue was first filtered through a 0.22 μ Millipore to exclude everything but virus-sized particles.

Viruses have been associated with a number of fish tumours[6], but this would appear to be the first report of transmission by cell-free filtrates of a malignant neoplasm found regularly in a natural fish population. The results of these experiments will be reported in greater detail elsewhere. Further work to characterize the neoplasm and to clarify the aetiology is in progress[7].

Zusammenfassung. Beim Hecht *Esox lucius* L. liess sich ein Lymphosarkom durch zellfreie Filtrate übertragen und damit die Virus-Aetiologie nachweisen.

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Lactate Dehydrogenase Virus Association with Transplantable Murine Tumors[1]

Since first described by RILEY et al.[2], the lactate dehydrogenase virus (LDV) has been reported in association with more than 50 types of transplantable murine tumors. Primary neoplasms, however, have usually been found to be free from it and certain tumors have been shown to contain the virus in one laboratory but not in another[3]. Thus, there appears to be little likelihood of an etiologic relationship to the neoplastic process.

The foregoing evidence to suggest that LDV is not involved directly in the production of murine neoplasms raises several questions of possible relevance. Among these, one of the more important may be stated as: do tumors activate the virus in carrier mice or is it introduced by accidental contamination during transplantation[3–7]? This paper will present results in support of the former alternative.

Materials and methods. Animals. Male and female C3H/Fg mice, approximately 3 months old, were used throughout the study.

Pretesting. 1 week prior to treatment, blood was collected from each animal in heparinized tubes. The specimens were then centrifuged at 1500 × *g* for 5 min and the resulting plasma samples assayed for lactate dehydrogenase (LDH) activity as described previously[8]. Without exception, the enzyme levels recorded were those of normal mice (i.e., between 300 and 1200 U/ml).

Tumors. Fibrosarcomas were induced by a single s.c. injection of 0.125 mg of methylcholanthrene in 0.2 ml of sesame oil.

Transplantation. Fresh tumor specimens were homogenized in a Ten Broeck grinder to give a 10% cell suspension in Eagle's basal medium. Each homogenate

was then injected s.c. (0.2 ml/mouse) into 3 pretested animals.

LDV. To determine the presence of LDV in primary and transplanted neoplasms, pieces of the tumors were removed, weighed, and homogenized (1 g of tissue: 9 ml of ice-cold Eagle's basal medium) for 1 min at 8000 rpm in a Servall Omni-Mixer. The homogenates were centrifuged for 10 min at 10,000 × *g* (0 °C), and the supernatants passed through Millipore filters of 220 nm pore size. Routinely, 3 pretested mice received an i.p. injection (0.1 ml/animal) of each filtrate, but, in some cases, as many as 10 animals were employed. 1, 3, and 5 weeks later, blood was obtained by tail bleeding; plasma LDH activities were measured spectrophotometrically[8].

Results and discussion. As shown in Table I, none of the methylcholanthrene-induced tumors (MIT 1 – MIT 14) tested for the presence of LDV was found to be positive. This observation is consistent with those of other investigators[3]; it therefore provides further evidence to indicate that primary murine neoplasms rarely contain the virus.

Of the 7 tumors which were followed through serial passages: a) 4 were still negative in the first (MIT 6), 4th (MIT 7), and 2nd (MIT 10, MIT 14) transplant generations; and b) 3 (MIT 11, MIT 12, MIT 13) were positive after the 3rd, 10th, and 8th transplant generations respectively (Table II). Admittedly, these results could be explained on the basis of accidental contamination. However, in view of the steps which were taken to minimize this possibility (e.g., all animals were pretested for the virus and sterile procedures were employed at all times), a more likely interpretation would seem to be that LDV, present in a latent state in some recipient mice, was activated by the tumors. Thereafter, each tumor served as a source of infection upon transplantation to other animals.

During the past few years, there have been reports of the effects, including impaired enzyme clearance[9] and altered immunologic response[10], which LDV may have on the host. Further, sufficient evidence has accumulated to suggest that these effects can also affect the interpretations of experiments with transplantable mouse tumors[3,11]. It is, then, in this connection that the above results seem of particular significance since they indicate that an accurate assessment of the presence or absence of LDV is not possible unless one tests systematically each transplant generation of a given murine neoplasm.

Table I. Tests for the lactate dehydrogenase virus in association with 14 primary tumors of mice

Tumor[a]	No. of recipient mice[b]	Mean plasma lactate dehydrogenase (U/ml)		
		1 week	3 weeks	5 weeks
MIT 1	3	900	–	900
MIT 2	8	500	850	600
MIT 3	3	900	750	550
MIT 4	7	500	–	900
MIT 5	4	650	900	850
MIT 6	5	–	950	950
MIT 7	3	850	850	700
MIT 8	5	–	500	850
MIT 9	5	750	550	600
MIT 10	3	850	850	700
MIT 11	3	850	800	800
MIT 12	3	–	350	600
MIT 13	3	650	650	850
MIT 14	3	750	850	900

[a] MIT, methylcholanthrene-induced tumor. [b] Each animal received an i.p. injection of 0.1 ml of cell-free tumor extract.

Table II. Tests for the lactate dehydrogenase virus in association with 7 transplantable tumors of mice

Tumor[a]	Transplant generation	No. of recipient mice[b]	Mean plasma lactate dehydrogenase (U/ml)		
			1 week	3 weeks	5 weeks
MIT 6	1	10	900	600	550
MIT 7	1–4	35	600	600	800
MIT 10	1–2	10	850	450	750
MIT 11	1–3	9	850	650	700
	4	3	4400	4250	–
	5	3	2850	3900	–
MIT 12	1–10	30	500	800	650
	11	3	3650	4400	4100
	12	3	3750	3300	–
MIT 13	1–8	24	800	900	900
	9	3	2450	2900	–
	10	3	3800	4100	3300
MIT 14	1–2	6	650	850	700

[a] MIT, methylcholanthrene-induced tumor. [b] Each animal received an i.p. injection of 0.1 ml of cell-free tumor extract.

Résumé. L'association du virus lactate déhydrogénase et des tumeurs de souris transplantables, semble être attribuable à une activation plutôt qu'à une contamination accidentelle. Cette observation, soutenue par de récentes études montrant que le virus peut affecter l'interprétation des résultats, fait ressortir la nécessité de vérifier chaque génération d'un néoplasme donné.

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