

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 described¹. Tumours are sited in many organs in the body but most often in the jaws. Viral aetiology has been suggested². 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 disrupted⁵, 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 tumours⁶, 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 progress⁷.

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

MAIRE F. MULCAHY and ANN O'LEARY

Department of Zoology, University College,
Cork (Ireland), 22 January 1970.

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² R. F. NIGRELLI, Zoologica 32, 101 (1947).

³ K. M. SMITH, quoted by MULCAHY (1963).

⁴ G. WINGQVIST, personal communication.

⁵ M. F. MULCAHY, Proceedings IVth International Symposium on Comparative Leukaemia Research (1969) in Bibliotheca Haematologica (Karger, Basel), in press.

⁶ K. WOLF, Adv. Virus Res. 12, 35 (1966).

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Lactate Dehydrogenase Virus Association with Transplantable Murine Tumors¹

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 another³. 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 transplantation³⁻⁷? 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 $\times g$ for 5 min and the resulting plasma samples assayed for lactate dehydrogenase (LDH) activity as described previously⁸. 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