1 year, and the plaques made by the virus are still of the mutant type. The mutant has been designated as *sp* (= small plaque). Cross-neutralization of plaques has so far shown no antigenic difference between *sp* and the wild type.

sp virus harvested 8 months after the establishment of the cell line was tested for tumour induction *in vivo*. In contrast to a 100 per cent incidence of animals with tumours after inoculation of wild type polyoma into hamsters, even with an input of 1·0–3·0 × 10^5 plaque-forming units of *sp* per animal, only subcutaneous sarcomas were found in 3 out of 31 hamsters (Table 1). One litter of *AKR* mice was inoculated when 2 days old with *sp*, and during an observation period of 6 months, parotid tumours only were observed in 1 out of 5 animals, in contrast to a 100 per cent tumour induction after inoculation of wild type virus.

Table 1. Tumour Induction in Hamsters after Inoculation with Wild Type Polyoma Virus and the Mutant *sp*

Virus	No. of litters	Age at inoculation (days)	Input virus per animal (plaque-forming units)	Observation period (months)	No. with tumour / No. of animals
Wild type	6	3 to 6	1·0 × 10^5	2·5	30/30
sp	3	2 to 4	1·0 to 3·0 × 10^5	2·5	1/16
	3	3 to 6	1·4 to 2·0 × 10^5	3 to 4	2/15

Animals inoculated subcutaneously with 0·1 ml. of virus suspension.

These results show that it is possible to obtain, with a tumour virus, a mutant that is 'attenuated' regarding its tumour-producing capacity, while retaining its cytopathic effect. The existence of complete attenuation for tumour-producing capacity, and of the reverse type of mutant, that is, tumour production without cytopathic effect, is now being looked for. It is of interest that in common with the *d*[4] and *MS*[5] mutations of polyomyelitis that can be associated with reduced neuropathogenicity, the reduced tumour induction by this polyoma mutant is also associated with a plaque type mutation.

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Virus and Antibodies in the Lymph and Blood of Sheep infected experimentally with the Virus of Tick-borne Encephalitis

It is known that foreign substances which penetrate the organism are resorbed by the lymphatic system. This also applies to bacteria and viruses.

In previous experiments[1] it was found that when tick-borne encephalitis virus was administered subcutaneously to mice, it was resorbed via the regional lymph vessels into the blood stream, from which it afterwards penetrated further lymph vessels and other organs. This was demonstrated by detecting the virus in the lymph nodes and blood.

Since I was interested in the question of the part played by the lymphatic and blood vascular systems in direct resorption of the virus from infected tissue, in their time relationships and in the antibody response in the lymphatic system, it was necessary to increase the accuracy of the preceding experiments by examining more adequate systems, namely, lymph and blood.

The experiments were carried out in sheep with an artificial lymph-blood circulation[2]. Lymph was collected from the cannulated thoracic duct and blood was obtained from a contralateral vein (one of the branches of the right jugular vein). The virus was detected in mice by intracerebral infection. Neutralizing antibodies were determined by an intraperitoneal test in mice.

The experiments clearly demonstrated that the lymphatic system is the main route by which the virus penetrates the blood stream from the subcutaneous tissue. No direct resorption of the virus from the subcutaneous tissue into the blood stream was found. If this occurs at all, it is evidently on such a small scale that it cannot be detected by the method used. The rate of resorption by the lymphatic system depends on the amount of virus. When administered subcutaneously into the metatarsus of the hind limb in amounts of 0·043 ml. 20 per cent suspension/1 kgm. body-weight (about three to four thousand million intracerebral mouse *LD*50), the virus was detected 60 min. later in lymph collected from the thoracic duct. No penetration of the virus into the blood stream from any part of the lymphatic system other than the thoracic duct was observed. Following re-infection seven and twelve days after primary inoculation, the virus was not detected in the lymph or the blood, even at very early intervals after reinfection[3].

Neutralizing antibodies were detected in the lymph sooner and in higher titres than in the blood plasma. Antibodies were also determined in the lymph of a sheep in which the thoracic duct was exteriorized and its connexion with the blood stream was interrupted, so that the virus, which was administered into a peripheral lymph vessel, passed through the regional lymph vessels only. This means that antibodies were determined in the lymph after antigenic stimulation to which, in effect, only the regional lymph vessels were exposed. The first antibodies were always found when the virus rapidly decreased in the lymph and in the blood. The higher antibody titre in the lymph was maintained up to the seventh day after infection. From then onwards it was higher in the blood plasma. Re-infection on the seventh and twelfth day after primary inoculation resulted in a further antibody increase in the lymph and the blood plasma, which was greater in the latter[4].

These experiments show that the lymphatic system plays an important part in resorption and transport of the tick-borne encephalitis virus and in the formation of antibodies against it.

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