fact does not contain principle adrenergic neurons. Regarding the catecholamine-uptake after application of L-DOPA, however, there is a significant difference between the nodose and the paracervical ganglion, as in the GPC there are cells able to take up catecholamine precursors, and these are either adrenergic neurons or they belong to the APUD system (SIF-cells in our case)[8]. Since in our study SIF-cells were purposely omitted, the cells capable of uptake were most likely adrenergic perikarya. In the GCS, there was a considerable decrease of IF after treatment with reserpine, while the mean IF of the GPC after reserpinisation did not differ significantly from that of the controls. This does not mean that the perikarya in the GPC as short neurons are more resistent to reserpine[9]; rather, the endogeneous catecholamine content in the GPC is very low.

In the superior cervical ganglion, there is an inverse relationship between the size of the perikarya and their mean IF[7]. In our study, the same relationship was observed, and in addition it also was found for partly depleted and repleted principle neurons of this ganglion, as well as for the control perikarya in the paracervical ganglia in spite of their low endogeneous IF. We suggest that this relationship is typical for adrenergic principle neurons, whether they belong to long or to short adrenergic neurons.

In figure 2 it is evident that the total amount of catecholamines in the GCS is higher than that in the GPC. The GCS is responsible for the innervation of metabolically very active organs like those of the head and part of the heart[10], whereas the GPC innervates comparatively inert organs like uterus, tube and ovary. It is well known that the catecholamine-contents of an adrenergic perikaryon is correlated to its nerve activity[11] which might possibly explain the low catecholamine-contents in the GPC. In the GCS there is a constant relationship between the IF before and after reserpinisation with decreasing size of the perikarya (table 3). This means that the larger perikarya release the same percentual share of catecholamines as the smaller ones upon systemic application of reserpine. Therefore, it can be assumed that reserpine distributes within the adrenergic perikarya in similar way as the endogeneous catecholamines. This relationship could not be found in the GPC, obviously because the initial values were too low and did not differ significantly from those of the reserpinised animals.

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Lymph node metastases of EMT6 tumour in nude mice

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Summary. Metastatic axillary lymph nodes following the injection of EMT6 tumour cells were observed in athymic nude mice, more often in female animals, and had a rapid growth rate. These metastases did not develop in syngeneic hosts. The latency of their appearance was inversely related to the number of injected cells.

It is well known that the prognosis of many types of human cancer is largely dependent on the presence of lymph node metastasis (LNM). It is thus justified to resort to experimental models in order the better to understand this type of spread[2]. The available models dealing with true LNM in mice usually consist of inoculating tumour cells in the foot pad[3-6]; intratibial[7], intratesticular[8], tail[9], thigh[5] and s.c.[10] injections have also been used. To our knowledge, none of these allowed a comparison of the characteristics of the initial tumour with those of LNM. Here we describe the occurrence of LNM in athymic nude mice after s.c. EMT6 cell injection with reference to sex differences and growth rate differences in relation to the primary tumour.

Materials and methods. The culture conditions of the EMT6 cell line and the technique of obtaining single cell suspensions have been described elsewhere[11]. Breeding and maintenance of athymic nude mice have also been previously reported[12]. From 1.3×10^2 to 7×10^5 cells in 0.1 ml were injected s.c. in the flank region of these mice. 7×10^5 EMT6 cells were also injected into female syngeneic hosts (BALB/c mice) as a control group.

Results. 5 days after the injection of 7×10^5 cells, a s.c. nodule became palpable having a doubling time (DT) of 2.5 days. This DT became longer as the tumour progressed in size, to reach 20 days before the death of the animals (figure 1). Homolateral axillary LNM started to appear at the beginning of the 5th week (figures 1 and 2) and had a DT of 1.1 day all through. The metastatic character of these

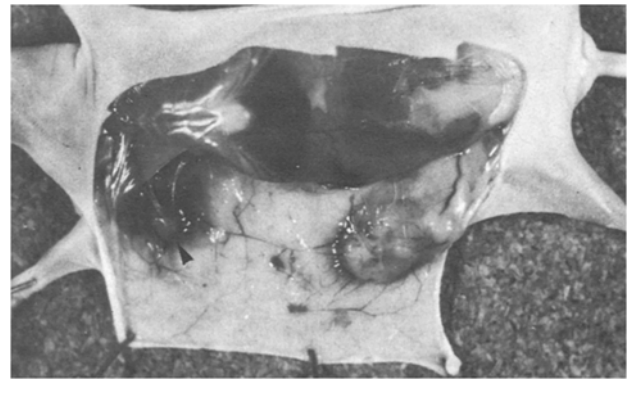

Fig. 1. Growth curves of EMT6 tumour in the s.c. region ○——○, and in the lymph node ●——●, following the injection of 7×10^5 cells.

lymph nodes was confirmed histologically. No direct continuity between s.c. tumours and LNM could be detected. Mediastinal LNM were sometimes observed and exceptionally contralateral nodes. The proportion of LNM was significantly higher in female than in male mice ($p < 10^{-6}$) (table). None of the injected BALB/c mice developed LNM. There was an inverse linear relationship between log number of injected cells (from 7×10^5 to 5×10^3 cells) and latency of appearance of LNM. For lower doses this time interval was relatively lengthened.

Discussion. The exclusive occurrence of LNM in nude mice may be attributed to their deranged immunological status, which is also probably responsible for the development of spontaneous lymphoreticular tumours rather than other types of tumours[13,14]. Sex is known to influence the development of metastases, especially with endocrine-dependent tumours[15]. It should be remembered that EMT6 tumour is a BALB/c mammary tumour. Moreover, gonadal dysfunction has been described in both male and female nude mice[16], however, other workers have not found any significant endocrinal imbalance[17].

Number of mice with lymph node metastases/number of mice injected subcutaneously with EMT6 cells

Females	298/499	(60%)
Males	33/103	(32%)
Total	331/602	(55%)

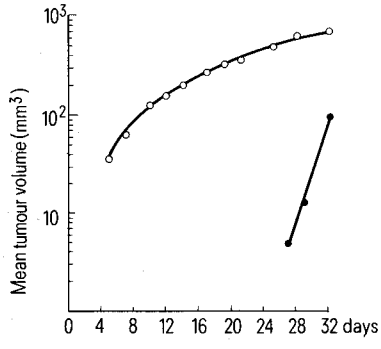

Fig. 2. A nude mouse with a s.c. tumour and a metastatic axillary lymph node (arrow).

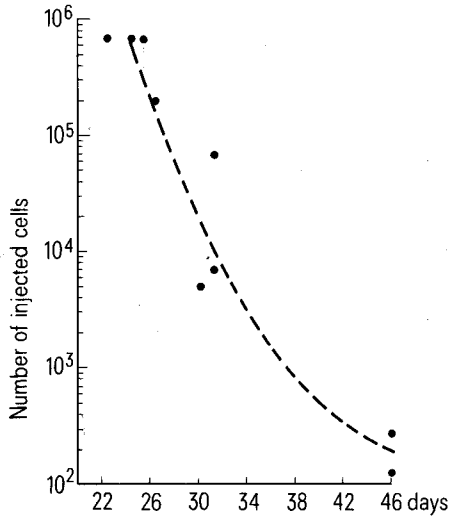

Fig. 3. Relationship between the number of injected cells and the appearance of the first palpable lymph node metastases. Each point was obtained by injecting between 25 and 30 mice.

The growth rate of LNM is about twice as rapid as that of a s.c. tumour of the same size. It is a priori unlikely that the lymph node milieu should be more favourable than the s.c. space for tumour growth. Cells growing in lymph nodes may derive from pre-existing variants in the initial tumour, for it has been found in some experimental systems that the parental tumour is heterogeneous regarding its metastasizing ability[18]. Moreover, it is probable that, at the time LNM appear, the immune system is exhausted due to the presence of a bulky s.c. tumour. It is also not excluded that a continuous flow of tumour cells from the s.c. site via lymphatics may contribute to the accelerated growth of LNM. Whatever the cause, we have recently reported another difference between s.c. tumours and LNM, the latter being more radioresistant[11].

As is shown in figure 3, there is a difference of about 3 weeks between the appearance of LNM following the injection of 7×10^5 cells and that following 1.3 or 3×10^2 cells. Growth curves of s.c. tumours at various challenging doses have revealed that the same difference in time (3 weeks) exists for tumours to reach 100 mm^3 following the injection of 7×10^5 and 3×10^2 cells[19]. If we assume that the DT of tumour cells in the lymph nodes before macroscopic detection is 1.1 day, which is supported by the slope of the exponential part of the curve in figure 3, it is likely that, after the injection of 7×10^5 cells, the first metastatic cell has started proliferation in the lymph node after the end of the 1st week at the earliest. This suggests the presence of a threshold for establishment of LNM[20].

The finding that LNM in this system is a late event, together with the observation that is does not occur in all injected animals, moves us to recommend the use of this model, among others, to study the immunological conditions associated with lymphatic spread and tumour cell arrest in lymph nodes, as well as the kinetics and therapy of metastatic involvement at these sites.

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