PATHOLOGY

Induction of Malignant Lymphomas by Urethane in Adult Mice bearing Syngeneic Thymus Grafts

In recent years it has repeatedly been proved that neonatal or very young animals are very susceptible to carcinogenesis induced by different agents. One possible explanation is that tissues of new-born animals contain a large number of incompletely differentiated cells, particularly responsive to carcinogenic stimuli. It is relevant to this speculation that a close correlation has been noted between susceptibility of mice to develop malignant lymphomas and the presence in the thymus of a great concentration of immature cells, whether by reason of early age[1], genetic constitution[2], whole-body X-irradiation[3], or regenerative changes in implanted thymus[4]. In thymectomized, virus-inoculated[5] or X-irradiated[4] mice, implantation of new-born thymus proved much more effective than adult thymus in restoring leukaemogenesis.

Previous work showed that urethane (ethyl carbamate) in adult mice was practically devoid of leukaemogenic activity[6,7], although effective in inducing thymic changes[8]. On the other hand, administration of this chemical to new-born animals was found to provoke a significant number of malignant lymphomas[6,9]. Urethane-induced lymphomas originated in nearly all cases in the thymus, only later acquiring the character of a generalized disease.

The experiments reported here were undertaken to investigate whether, by making use of single or multiple grafts of new-born thymus as a more suitable target tissue, adult mice would be rendered susceptible to the leukaemogenic action of urethane.

C57BL male mice aged 2–3 months were grafted subcutaneously into the right flank with one or five thymuses obtained from 1- to 5-day-old syngeneic donors. Starting 5 days after implantation, they received urethane in drinking water at the 0·3 per cent level for 30 days, continuously. Adult *C57BL* mice of both sexes treated only with urethane served as controls. The total quantity of the chemical administered to animals of the different groups amounted to an average of 220 mg per mouse. A certain number of mice died within 30 days from the end of treatment, presumably owing to the toxic effect of urethane. They were not included in the investigation.

Table 1. Malignant Lymphomas induced by Urethane in *C57BL* Mice implanted with Syngeneic Single or Multiple Thymuses

Thymus graft	No. of mice in group	Mice with lymphomas No.	Mice with lymphomas %
None	44	1	2
1 thymus	15	2	13
5 thymuses	22	9	41

The results, which refer to an 8-month period of observation from the end of urethane administration, are summarized in Table 1. It is evident that the highest incidence of malignant lymphomas occurred in the group grafted with 5 thymuses (41 per cent), while in animals receiving only a single thymus the incidence was markedly lower (13 per cent). However, only in the case of multiple grafts was the incidence statistically significant in comparison with the control group ($\chi^2 = 14{\cdot}15$; $0{\cdot}001 < P < 0{\cdot}01$). Neoplastic disease in all thymus-implanted animals was initiated after a mean latent period of four months as a localized growth at the graft site, and reached a maximum diameter of 40–50 mm in about 40 days (Fig. 1). Generalized leukaemia appeared later, except in three animals implanted with 5 thymuses, which at death had only local tumours. *In situ* thymus of leukaemic mice, although appearing grossly of almost normal size, showed histologically, in all cases in which the organ could be recognized with certainty, initial or limited neoplastic changes. Lymphomatous tissue at the site of implantation, as well as in the various organs involved by the generalized disease, was composed of cells of lymphoid type.

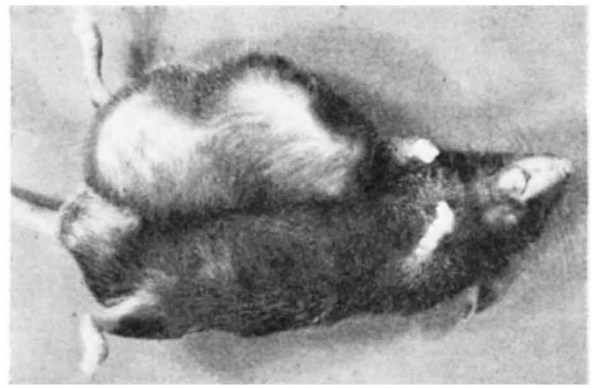

Fig. 1. *C57BL* mouse implanted with multiple syngeneic new-born thymuses and treated with urethane in drinking water. Large neoplastic mass at the graft site, 130 days after the end of urethane administration

Present data suggest that grafts of neonatal thymus make susceptible to the leukaemogenic action of urethane the otherwise resistant mice. Since in our experiments lymphomas constantly arose from the implanted thymus(es), it is possible that the positive results obtained in both the experimental groups are correlated with the availability in the graft of cells particularly susceptible to urethane-induced damage leading to neoplasia. On the other hand, the finding of the highest susceptibility in the group implanted with multiple thymuses seems to indicate that a quantitative factor is involved in the process of neoplastic transformation of the graft. Thus, it could be assumed that in a single thymus graft the number of cells sensitive to the weak leukaemogenic activity of urethane scarcely reaches the critical level necessary to initiate successfully a lymphomatous growth, under the present experimental conditions. However, the mechanism through which a greater mass of thymic tissue influences leukaemogenesis, that is, by contribution of more target cells and/or production of larger amounts of humoral substance, remains to be elucidated.

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