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COMPARISON OF CLONOGENIC CELL ASSAYS AFTER *IN VIVO* AND *IN VITRO* TREATMENT OF 9L GLIOSARCOMA

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THE simultaneous application of different methods to quantitate the response of individual tumour lines provides complementary information that will lead to a better understanding of the effects and limitations of a therapy.

Cells of 9L gliosarcoma were grown as solid brain tumours in Fischer 344 rats (Rosenblum *et al.*, 1975, *Cancer Res.*, **35**, 1387) and as monolayers and spheroids in culture. Single cells were analysed for clonogenic capacity by a colony-forming assay before and after *in vivo* and *in vitro* treatment with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU), 1-(2-chloroethyl)-3-(2,6-dioxo-3-piperidyl)-1-nitrosourea (PCNU) and radiation therapy.

For 9L brain tumours *in vivo* (50-100 mg), monolayer cultures (asynchronous, exponential growth), and spheroids (200–500 μm diameter), the respective cell cycle times indicated by autoradiography were 19·3, 19·8 and 20·4 h; the DNA synthesis phases were 7·6, 8·2 and 8·3 h; the growth fractions were 0·51, 0·97 and 0·52. The colony-forming efficiency (CFE) of untreated single cells disaggregated from solid tumours, monolayer cultures, and spheroid cultures were, respectively, 10–30%, 70–80% and 40–50%. After well established 9L tumours were treated *in vivo* with BCNU the tumour cell kill and the

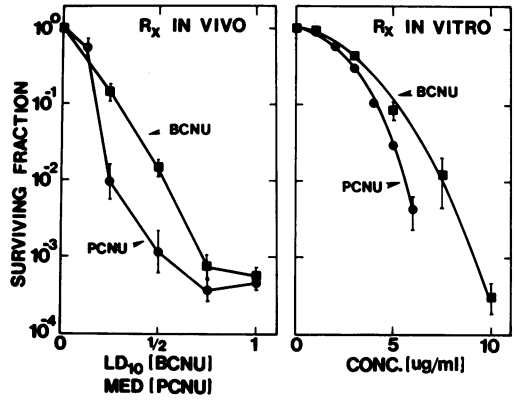

FIGURE.—Cell survival following treatment of 9L brain tumours *in vivo* and of monolayer cultures *in vitro*. The *in vivo* dose is represented as a fraction of the LD_{10} (13·3 mg/kg) for BCNU and the maximally effective dose (MED = 25 mg/kg) for PCNU so as to relate tumour cell sensitivity to host toxicity as a representation of the therapeutic index of each agent.

TABLE.—*Tumour cell survival following* in vitro *and* in vivo *treatment of 9L gliosarcoma with BCNU and irradiation*

Treatment			Log cell kill			
			In vitro			
	Dose*			Spheroid		
Agent	*In vitro*	*In vivo*	Monolayer	Rx as cells	Rx as spheroid	*In vivo*
BCNU	3 μg/ml	4·1 mg/kg	0·36	0·46	1·47	1·05
	6 μg/ml	8·2 mg/kg	1·28	2·82	3·96	2·33
Radiation	10 Gy	10 Gy	0·96	0·67	0·52	0·88†
	15 Gy	15 Gy	1·85	1·52	1·24	1·58†
	20 Gy	20 Gy	2·92	2·47	2·02	2·30†

* Doses for equivalent tumour cell exposure *in vitro* and *in vivo* (V. A. Levin, personal communication).
† From Leith *et al.*, 1975, *Cancer*, **35**, 1545.

time to repopulation of the surviving clonogenic cell pool correlated with increased animal lifespan (Rosenblum *et al.*, 1976, *Cancer Res.*, **36**, 3718). These observations confirm that the CFE assay is a true measure of *in vivo* antitumour activity. The cell-survival curves obtained after *in vitro* treatment of solid tumours or *in vitro* treatment of monolayer and spheroid cultures with BCNU, PCNU, or irradiation appeared similar (Fig. and Table).

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