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BIOLOGICAL EFFICACY OF A BORONATED PORPHYRIN AS MEASURED IN CELL CULTURE

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DISCLAIMER

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Of all the various biomolecules being evaluated as vehicles for boron transport to tumor, porphyrins have the highest boron-carrying capacity on the basis of weight. Further, they are known to be taken up robustly by the many types of tumors evaluated to date. Porphyrins suggest themselves immediately for application in NCT of brain tumors, as they clearly do not penetrate the blood-brain barrier, thus providing, in principle, a physical therapeutic gain.

Attempts to develop a boronated porphyrin analog have suffered from problems associated with toxicity, solubility and, upon occasion, lability of the boron tag. One compound synthesized by one of us (SBK), designated "SBK-II", has evidently overcome the above problems, providing therapeutic amounts of boron in animal tumor models (20-40 μg B/g tumor) with retention times in the order of weeks. As is well known, biological efficacy will depend upon distribution within (or without) the cell. In the experiments detailed here, biological efficacy has been evaluated in Hamster V-79 cells, irradiated at the thermal neutron beam at the Brookhaven Medical Research Reactor, using irradiation geometries and techniques as described in detail elsewhere (1). Cell survival curves obtained with 15 and 29 μg ^{10}B per ml from $H_3{}^{10}BO_3$ ("ambient" conditions) were compared to a control (no boron) curve (data not shown). The average thermal neutron fluence rate in the cell suspension is 2.8×10^{11} cm^{-2} min^{-1}) with photons and fast neutrons providing an adventitious dose rate in water of 6.5 and 13 rad/min, respectively, (Reactor Power 1 MW; for higher ^{10}B concentrations, lower power was used). When the inverse of the Do's is plotted vs ^{10}B concentration, a straight line is obtained from which the equivalent uniform boron (H_3BO_3) concentration can be obtained from an

unknown boron distribution (1). These procedures are presented in detail in Ref. 1.

Both monomer and dimer forms of the sulfhydryl boron hydride ($Na_2B^1{}_2l_2H_{11}SH$) were irradiated, with cells suspended in ≈30 μg $^{10}B/g$ of compound ("ambient"), and after being washed 3 times in PBS and resuspended in boron-free medium ("washed"). Results for monomer and dimer are displayed in Figs. 1 and 2 respectively. As can be seen, washing removes most of the boron, while irradiation in ≈30 μg $^{10}B/ml$ of "ambient" boron produces an effect equivalent to 9.5 and 12 μg $^{10}B/g$ of H_3BO_3 (uniform distribution) for monomer and dimer, respectively (Table I). Clearly, the intracellular B concentration is less than the ambient concentration of ≈30 μg $^{10}B/g$.

When cells incorporating boronated porphyrins are irradiated (compound SBK-II, as obtained from S.B. Kahl), survival curves for ≈30 μg/ml "ambient" boron and for washed cells show an enhanced response (Fig. 3). As listed in Table I, equivalent boron concentrations are 40 and 12 μg $^{10}B/ml$ for "ambient" and "washed" experiments. The 40 μg B/g figure indicates that the cells somehow "concentrate" the porphyrin. The second concentration (12 μg/g) suggests that if the porphyrin is intracellular, washed cells have less than 30 μg ^{10}B distributed uniformly throughout the cell, but still more than 3x as much as obtained under similar conditions with BSH and BSSB.

Calculations clearly show that if hamster V-79 cells are irradiated with boron bound to the cell membrane, or in an extracellular location, ≈ 10-15x as much is needed to produce the same response obtained when boron is uniformly distributed within the cell (2). Since location is of such importance with respect to required boron concentrations, samples of ≈ $2x10^8$ cells were grown for 12 hours in the presence of porphyrin and dimer, and then washed prior to

prompt-γ boron analysis. Results are shown in Table I (28 $\mu g^{10}B/g$ per porphyrin, and 6,2 μg $^{10}B/g$ for BSSB), and demonstrate that boron is located intracellularly (otherwise $\approx$ 110 and 35 μg $^{10}B/g$ would have been needed for porphyrin and BSSB, respectively, to obtain the observed radiobiological response (2). In view of the approximately equal contribution for boron located in the nucleus or cytoplasm (with equal local concentration per unit volume (2), the above data are consistent with a cytoplasmic location.

SUMMARY

The advent of boronated compounds such as SBK-II with an intracellular localization greatly enhances the efficacy of the boron reaction for NCT. The in vitro system used in this evaluation can answer questions such as transport mechanisms, cellular distribution, toxicity levels, dose, etc. It is the in vitro system which is cost-effective in terms of the precious lives of animals and the amount of compound required to establish efficacy and additionally provide the most rapid results. It is the in vitro system which demonstrated the intracellular incorporation of SBK-II and its biological effectiveness as being three times more effective than BSH or BSSB.

The technique described above compares biological efficacy in cell cultures with a uniform distribution of H_3BO_3. It is capable of providing information about cellular localization and at this time is the only technique which has been used successfully for this important parameter.

REFERENCES

1. Gabel, D., Fairchild, R. G., Borner, H. G., and Larsson, B. The Monte Carlo simulation of the biological effect of the $^{10}B(n,\alpha)^{7}Li$ reaction in cells and tissue and its implication for boron neutron capture therapy. Radiat. Res. 98, 307-316 (1984).

2. Gabel, D., Foster, S., and Fairchild, R. G. The relative biological effectiveness in V79 Chinese Hamster cells of the neutron capture reactions in boron and nitrogen. Radiat. Res. 111, 14-15 (1987).

TABLE I

RESPONSE OF V-79 CELLS TO THERMAL NEUTRON IRRADIATION, FOLLOWING GROWTH FOR 12 HR (1 CELL CYCLE TIME) IN THE PRESENCE OF 30 $\mu g^{10}B/g$ FROM VARIOUS COMPOUNDS

	Do(min)		Boric Acid Equivalents ($\mu g/g$)		Measured B Content ($\mu g/g$)
COMPOUND	WASHED	AMBIENT	WASHED	AMBIENT	
BSH	3.3	1.4	0.5	9.5	
BSSB	2.2	1.2	3.5	12	6.2
SBK-II	1.2	0.5	12	40	28

Legends for Figures

Figure 1:

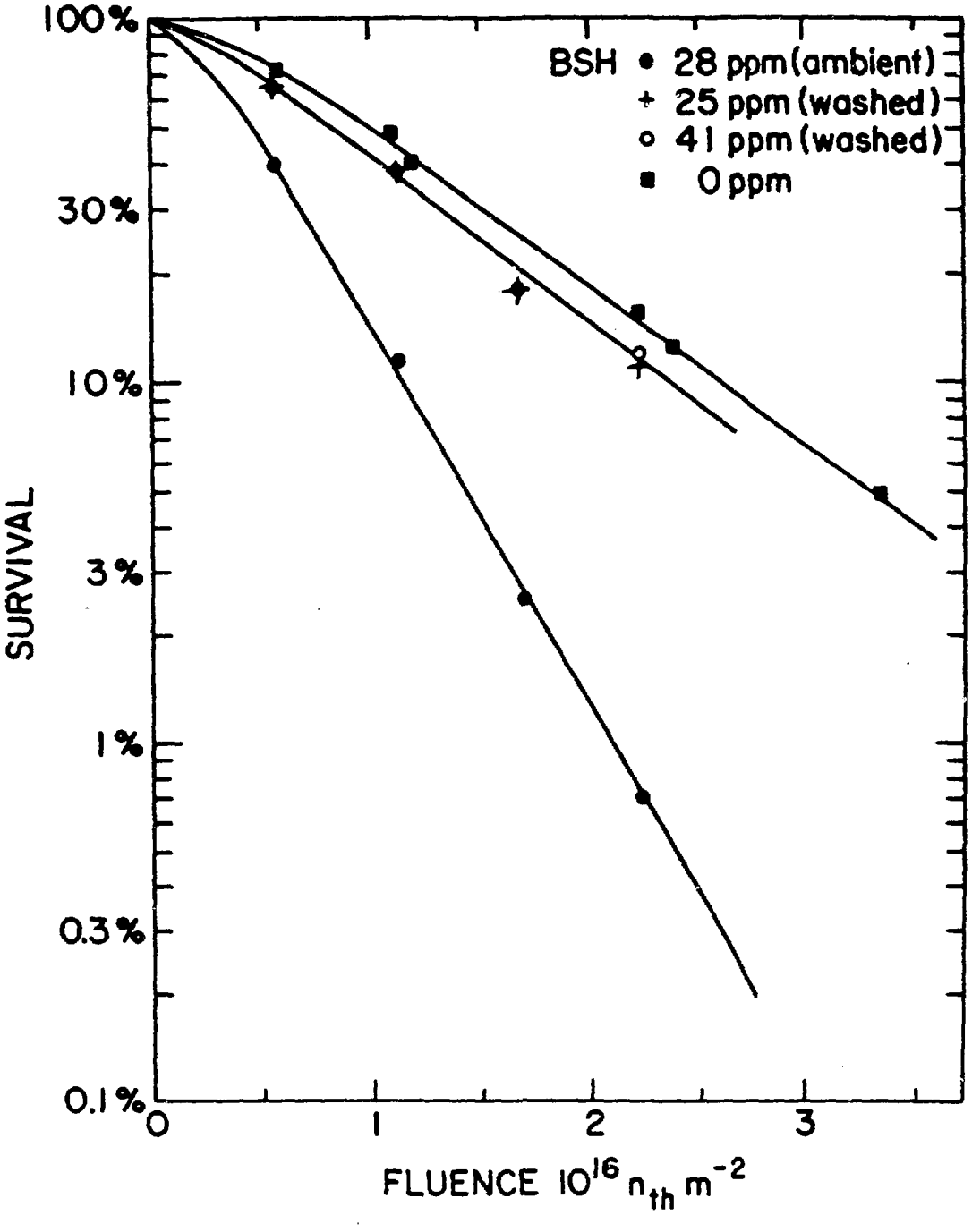
Reproductive death of hamster V-79 cells grown for 12 hours in the presence of BSH: and exposed to thermal neutrons.

Figure 2:

Reproductive death of hamster V-79 cells grown for 12 hours in the presence of BSSB: and exposed to thermal neutrons.

Figure 3:

Reproductive death of hamster V-79 cells grown for 12 hours in the presence of boronated porphyrin (SBK-II): and exposed to thermal neutrons.


100%
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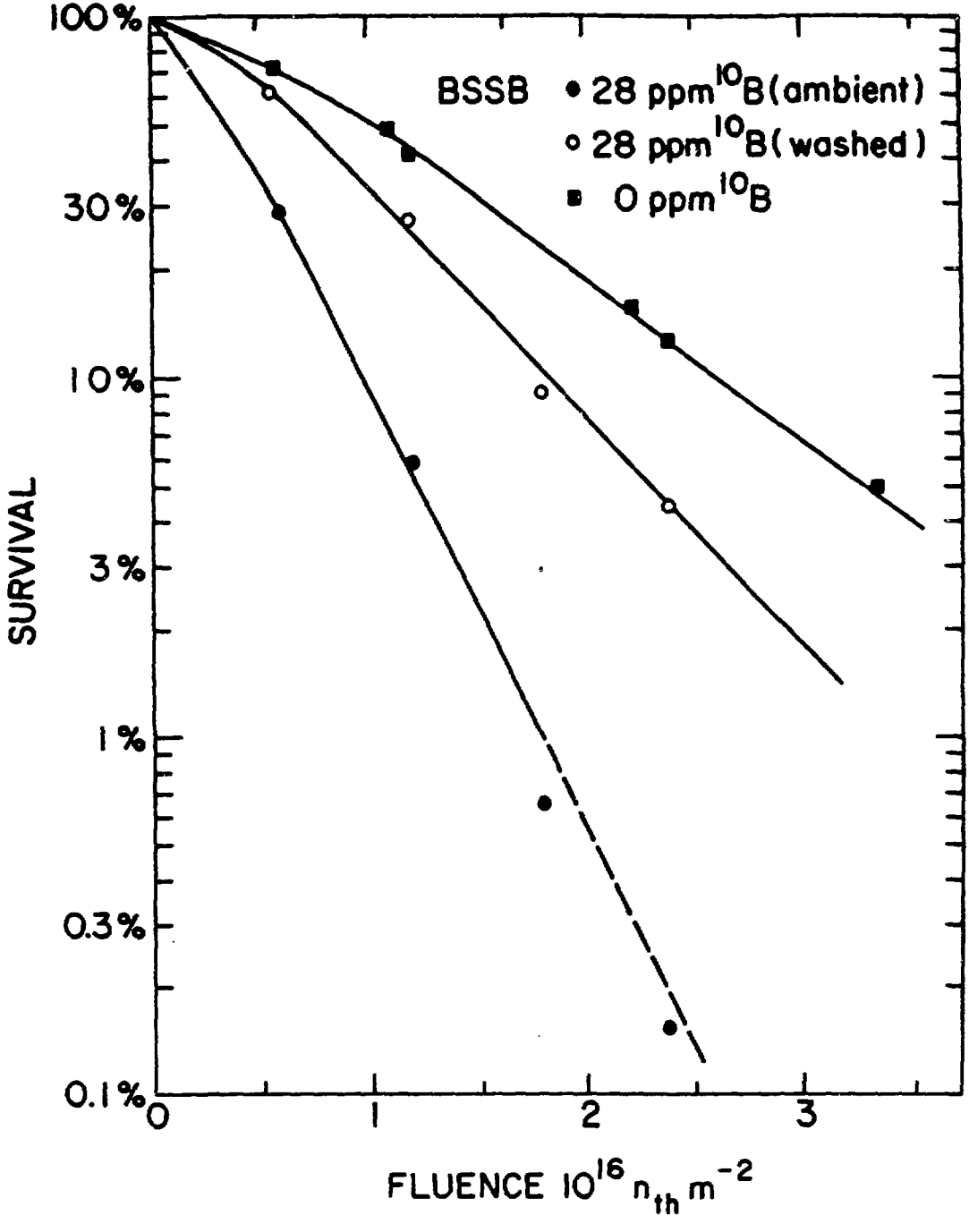
SURVIVAL
BSH • 28 ppm (ambient)
+ 25 ppm (washed)
o 41 ppm (washed)
■ 0 ppm
0
1
2
3
FLUENCE 10^{16} n_{th} m^{-2}

FIGURE 1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SURVIVAL
BSSB
• 28 ppm ^{10}B (ambient)
○ 28 ppm ^{10}B (washed)
■ 0 ppm ^{10}B
0
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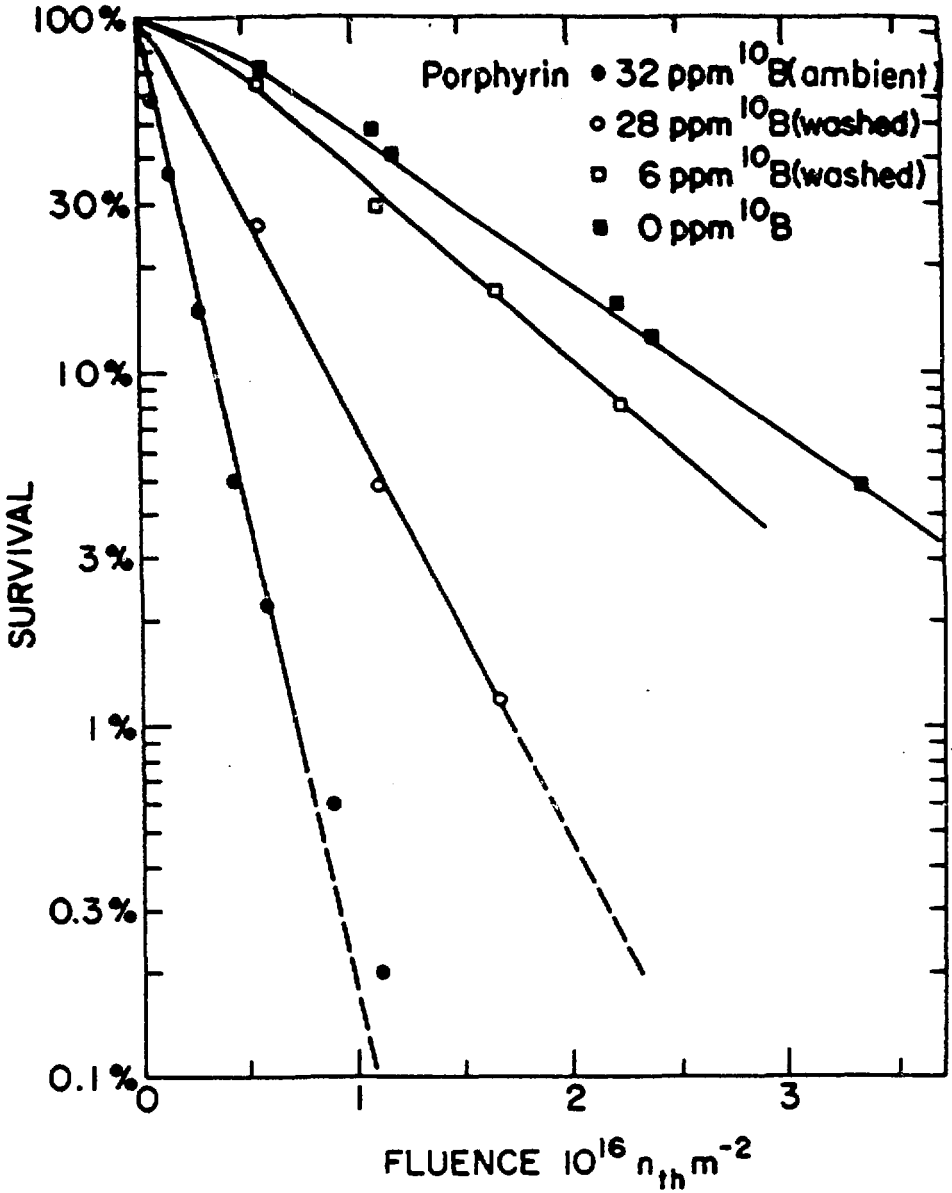
2
3
FLUENCE 10^{16} n_{th} m^{-2}

FIGURE 2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0.1%
SURVIVAL
Porphyrin • 32 ppm ^{10}B(ambient)
○ 28 ppm ^{10}B(washed)
□ 6 ppm ^{10}B(washed)
■ 0 ppm ^{10}B
0
1
2
3
FLUENCE 10^{16} n_{th} m^{-2}

FIGURE 3