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6. F. Mosteller and J. W. Tukey, data analysis including statistics, in *Handbook of Social Psychology*, G. Lindzey and E. Aronson, Eds. (Addison-Wesley, Reading, Mass., 1968) vol. 2, pp. 133–160; M. Stone, *J. R. Statist. Soc. Ser. B* (1974), p. 111.
7. The L1 and L3 anesthesia level EEG's are likely not to be equally well classified. The overall probability of classification error is minimized by the KL-NN rules independent of the prior possibilities on costs for different classification errors (*1*). The statistical properties of the KL-NN and KL-*k*NN rules (*3*, *4*) imply that the best estimate of the achievable discrimination performance is obtained by the best performing KL-*k*NN rule.
8. McEwan studied anesthesia level EEG classification using spectral analysis-discriminant analysis methods on the same data base described in the text. Sixty-five and 80 percent correct classification performance on L1 and L3 anesthesia level EEG's was achieved with 13 and 26 prespecified discrimination features, respectively (*2*). McEwan's are probably the best previous EEG clinical population screening results obtained.
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10. We used a time-shared PDP/1145 computer. For $d = 2$ ($d = 4$) EEG data channels, 853 sample/channel time series, computational steps (i) and (ii) each required approximately 20 (90) seconds. A brute force approach to the computation of step (iii) required 1.2 (4.8) minutes. Our FORTRAN programs for those computations are available upon request. Array processors connected to PDP 11 machines can implement those computations 100 times faster. Such parametric time-domain computations have been implemented for speech recognition research (J. Makhoul, Bolt, Beraneck and Newman, Cambridge, Mass., personal communication). For the $d = 1$ data channel those computations are done in tens of milliseconds. Chips to do steps (i) and (ii) for the $d = 1$ channel exist for real-time secure voice communication (C. S. Miller, C. F. Motley, R. A. Allen, in *EASCON-77*, IEEE catalog No. 77CH1255-9). Extension of the existing array processor implementations for the $d = 1$ channel to process an arbitrary number of channels is straightforward.
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13. This work was supported in part by NSF grant 74-09883 and Air Force Office of Scientific Research grant 78-3564 at the University of Hawaii.

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Hyperthermia and Local Anesthetics: Potentiation of Survival of Tumor-Bearing Mice

Abstract. *Lidocaine infusion of a CA755 mammary adenocarcinoma growing in the hind leg of BDF$_1$ mice results in a significant increase in the animals' survival when combined with heating for 1 hour in a 43.5°C water bath. This ability of local anesthetics to prolong survival following hyperthermia is consistent with the hypothesis that increases in membrane fluidity influence sensitivity to heat. In view of the extensive clinical experience with local anesthetics, the delay between clinical application and the observation that they potentiate the action of hyperthermia in animals may be reduced.*

This report describes the potentiation by an anesthetic of the tumor-inhibiting effect of local hyperthermia. The combined therapy resulted in apparent local cures in four of 31 animals.

In studies aimed at reevaluating the "lipoid liberation" theory of Heilbrunn (*1*), we obtained evidence in support of the hypothesis that the fluidity of membranes as determined by their lipid content is a major contributing factor in the death of cells exposed to hyperthermia. Singer (*2*) observed that a lower temperature was required for an increase in liposome permeability (^{22}Na efflux) when local anesthetics were present. Local anesthetics increase marker molecule mobility and fluidity in intact cell membranes and cell membrane fractions and increase the rotation of probe molecules dissolved in synthetic protein-free lipid bilayers (*3*). We have shown (*4*) that procaine-HCl increases hyperthermic killing of an unsaturated fatty acid auxotroph of *Escherichia coli*. Data obtained with V79 Chinese hamster lung cells (*5*) also show a potentiation of hyperthermic killing by procaine. On the basis of these observations, we postulated that local anesthetics might potentiate the therapeutic effect of hyperthermia in treatment of malignant disease (*4*).

Young adult BDF$_1$ mice grafted with mammary adenocarcinoma strain CA755 were used throughout. For transplantation, tumors were removed from donor mice and a crude suspension was prepared with the aid of a Snell cytosieve (*6*). When the tumors measured approximately 4 mm in mean diameter, the mice were randomly assigned to treatment groups. Tumor sizes were determined by caliper measurement of the maximum dimension. These data will be reported separately (*7*). Responses vary from disappearance of tumors to delays in growth to a reduced rate of growth after treatment.

Mice were anesthetized by intraperitoneal injection of 14 mg of chloral hydrate. They were placed on special carriers with the tumor-bearing leg drawn through an opening for immersion in a Tecam constant-temperature bath with a TU Tempunit circulating heater and a Yellow Springs Instrument telethermometer thermistor probe. The legs of the mice were gently held in place by masking tape over the lower portion of the limb during heating. The tumor-bearing legs were exposed to bath temperatures of 42° or 43.5°C (± 0.1°C) for 1 hour. Water surfaces were insulated with plastic spheres 2 cm in diameter both to aid in maintaining constant bath temperature and to further insulate the remainder of the animals' bodies from heat. The air temperature above the water bath at the level of the mouse carrier did not exceed 37°C.

Lidocaine-HCl (Elkins-Sinn, Inc.) was infused by injection into three areas of the tumor in a volume of 0.05 ml (2 mg per mouse) within 5 minutes before heat treatment. Some comparison groups were similarly infused with 0.05 ml of isotonic saline. In each experiment, nine groups of animals were randomly assigned to a 3 by 3 block design of treatment regimes with three temperatures [room temperature (22° ± 1°C), 42°, and 43.5°C] (*8*), and three injection options (no injection, saline injection, and lidocaine injection).

There was a significant interaction between lidocaine infusion and the temperature of heating in all three experiments thus far completed. Mean survival of the mice with lidocaine-infused tumors heated at 43.5°C for 1 hour was signifi-

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Table 1. Survival after treatment. Values are means ± standard errors; number of animals is shown in parentheses. Analysis of variance indicates that the increase in survival when lidocaine is present during heating at 43.5°C for 1 hour is significantly different ($P < .05$) from the results for the other eight treatments. Heat alone at 43.5°C significantly prolonged survival. The figure for lidocaine plus 43.5°C is minimal as it included four animals that were still alive.

Heating*	Survival (days) after injection		
	None	Saline	Lidocaine
None	12.8 ± 0.90 (25)	12.7 ± 0.70 (17)	14.6 ± 1.21 (17)
42.0°C	12.6 ± 1.36 (10)	16.5 ± 1.61 (10)	13.6 ± 0.89 (10)
43.5°C	18.5 ± 0.88 (31)	19.4 ± 1.08 (30)	> 37.3 ± 6.21 (31)

*For 1 hour.

cantly greater than that of all other comparison groups (Table 1). Four animals in this group are still alive, and the mean survival of > 37.3 days calculated is thus a minimum. The four animals still tumor-free and alive had survived for 90, 125, 130, and 131 days as of 18 December 1978. On gross examination at autopsy of mice treated with lidocaine at 43.5°C in which grafted tumors were not detectable, the muscle tissue at the transplant sites appeared healthy and normal. Lidocaine did not significantly alter mean survival at 42°C.

Heat at 43.5°C alone increased mean survival significantly, while heating at 42°C did not. These results confirm the earlier observation that relatively little effect of hyperthermia on cell survival is noted until temperatures above 42° to 42.5°C are attained (*9*). Lidocaine or saline alone was without significant effect. For each treatment, the fraction of mice that died each day after treatment and the cumulative fraction that survived were calculated. Under each of the three heating regimes, the cumulative survival curves for both the saline-injected mice and the mice that received no injection were essentially the same. These data were pooled for graphical presentation. An increase in survival in the lidocaine-43.5°C group is illustrated in Fig. 1. There are four of 31 mice surviving with apparently complete local control of tumors.

The effects of lidocaine greatly potentiated the effect of temperature. A major drug-induced increase in survival occurred only at 43.5°C, and there was a distinct interaction between the anesthetic and heat at this temperature. The survival increase is significant ($P < .05$) when compared to 43.5°C hyperthermia only, to treatment at 42°C, or to the groups not subjected to any heating. In one trial, bupivacaine (Marcaine) at a dose of 0.375 mg per mouse with heating at 43.5°C for 1 hour was compared with lidocaine and the increase in survival was almost identical in both cases. These findings are consistent with earlier predictions (*4*) based on the greater killing of *E. coli* when a hyperthermia regimen was combined with exposure to procaine. Both heat and local anesthetic reportedly disorganize (fluidize) membrane lipids (*1–3, 10*).

We believe that the interaction of local anesthetics with hyperthermia differs in cellular mechanism from reported interactions of cytocidal agents with hypothermia. For example, Hahn *et al.* (*11*) described an increase in uptake of adriamycin at 43°C as compared to 37°C, accompanied by a marked decrease in cell survival after adriamycin and bleomycin combined with heating at 43°C.

We do not believe the increase in survival after lidocaine and hyperthermia results from a decrease in tumor blood flow, with a consequent rise in local temperature. Chloral hydrate, the general anesthetic used, would be expected to reduce peripheral blood flow. In the presence of this type of general anesthetic agent, lidocaine might be expected to increase local blood flow by blocking the sympathetic nervous system (*12*).

When one considers that the CA755 tumor contains a high proportion of hypoxic and therefore radiotherapy-resistant cells (*6, 13*), the extension of survival by treatment with local anesthetic combined with exposure to water bath temperatures of 43.5°C is striking, as is the apparently disease-free survival of four treated animals for more than 90 days.

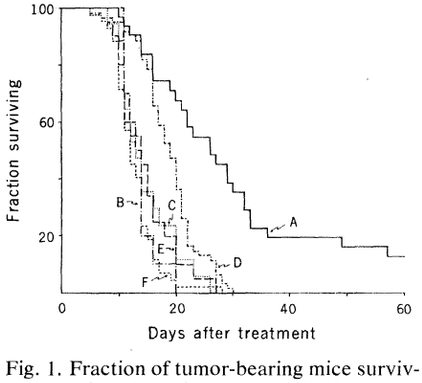

Fig. 1. Fraction of tumor-bearing mice surviving as a function of days elapsed after treatment for (A to C) groups receiving lidocaine and (D to F) groups receiving either saline injection or no injection. In groups A and D, the tumor-bearing legs were heated at 43.5°C for 1 hour; in B and E, they were heated at 42.0°C for 1 hour; and in C and F, they were not heated. Numbers of animals were (A) 31, (B) 10, (C) 17, (D) 61, (E) 20, and (F) 42.

The pharmacology of local anesthetics is well known, clinical experience with them is extensive, and they are readily localized in tissues. These factors may encourage their speedy clinical application in enhancement of hyperthermia.

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7. M. B. Yatvin, K. H. Clifton, W. H. Dennis, in preparation.
8. Based on published work on hyperthermia, heating at 43.5°C was expected to clearly show an increased survival, while heating at 42°C was closer to the expected threshold of the heating effect. As hyperthermia temperature was increased above the threshold temperature, we found that the increase in killing in *E. coli* with procaine decreased. This is consistent with the postulate that increased temperature and local anesthetics have additive effects mediated by their alteration of membrane fluidity. Further, raising the temperature above 43.5°C would increasingly destroy normal tissue. As our goal is to enhance therapeutic efficiency, we seek the lowest temperature where a significant cure can be achieved.
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