ABSTRACT

The treatment of intrahepatic or perihepatic neoplasms by hyperthermia may be limited by the thermal sensitivity of normal liver tissue. To establish the temperature dependence of hepatic toxicity, eight canine liver lobes were exposed to a single 30-min dose of localized hyperthermia in the range of 43.0°C-47.5°C, induced by radiofrequency currents. Four additional liver lobes were conditioned with a pretreatment dose of 43.0°C/30 min and challenged at either 44.5°C/30 min or 47.5°C/30 min, 4 h later. Temperature distributions were measured using implantable thermocouple sensors. Treated areas were sampled 28 days later, and liver damage was determined using histopathological criteria. Most treated sites showed only modest alterations. The parameters of tissue injury that correlated best with dose were: evidence of hepatocyte loss; focal fibrosis; and distortion of lobular architecture. Areas of necrosis were observed in several samples, but their presence or severity did not correlate with dose. Thermal damage to liver capsule, liver lobules, portal areas, and central veins did not exhibit monotonic dose-response relationships. The data do not demonstrate thermotolerance; in fact, they suggest, although do not prove, its absence. If thermotolerance did not develop, vascular effects might explain such a finding.

INTRODUCTION

The usefulness of hyperthermia as a clinical tool will depend upon our ability to adequately heat tumors without inducing excessive normal tissue damage. Rational treatment plans can only be designed to spare specific, heat-sensitive tissues, when the thermosensitivity of these tissues is determined, and when "acceptable" heat-induced damage is defined (1).

Protection of normal tissue by judicious induction of thermal resistance could permit tumor treatment, at higher thermal "doses." Germer and Schneider (2) and Henle and Leeper (3) demonstrated the phenomenon of transient protection of cells in tissue culture exposed to a two-fraction heat regimen. This phenomenon of transient protection against subsequent heat challenges has been termed thermotolerance and has been extensively studied both in vitro and in vivo (4, 5). The planned induction of thermotolerance in selected normal tissues could provide differential protection greatly enhancing the antitumor potential of hyperthermia.

In earlier communications, we reported on the thermal sensitivity of adipose tissue and skeletal muscle in large animals and on the effects of in vivo induction of the state of thermotolerance in these tissues (5, 6). It was shown that selective pretreatment can reduce the heat sensitivity of these tissues. A "therapeutic advantage" of about 2°C was observed when tissues were conditioned with a priming dose of 43°C/30 min, given 4 h prior to the challenging heat treatment.

In this paper, we report on the thermosensitivity and attempts to induce thermotolerance in the normal canine liver.

MATERIALS AND METHODS

Liver Heating. Four dogs, weighing between 20 and 30 kg each, were selected from a random pool of healthy kennel animals. They were fed standard diets and fasted for 24 h prior to the experiment. Animals were anesthetized with an i.v. injection of 9--25 mg of thiopental sodium per kg (Parke-Davis, Div. Warner-Lambert, Inc., Morris Plains, NJ) and maintained on halothane inhalation anesthesia (0.5--2%). The liver was exposed through a midline incision. The distal part of each lobe was heated by means of radiofrequency-induced currents (frequency = 0.5 MHz) flowing between a pair of parallel-plate disk-shaped electrodes placed across it (Chart 1A). The interelectrode distance varied from site to site in the range of 1.1--2.4 cm. Details of the heating apparatus can be found elsewhere (7).

Eight liver lobes were exposed to a single 30-min heat "dose" in the range of 43.0°C--47.5°C (Chart 1A). Four additional lobes were conditioned with a priming dose of 43.0°C/30 min and challenged at either 44.5°C/30 min or 47.5°C/30 min 4 h later. Site A (Chart 1A) was conditioned to 43.0°C/30 min and challenged at either 44.5°C/30 min or 47.5°C/30 min, 4 h later. Site B was sham treated. Site C received a single treatment of either 44.5°C/30 min or 47.5°C/30 min. Site D was not treated and controlled for anesthetic (halothane) injury. Site E received a single 43.0°C/30-min treatment.

Systemic temperature was monitored during the experiment by means of two thermocouple sensors. One sensor measured rectal temperature, and the other deep abdominal temperature, away from the heated site. The systemic temperature varied slightly during the experiment. The range of differences between starting and final body temperature was about 3°C. During the treatment, the steady-state values of current, voltage, and their phase angle difference were measured. Triple-point temperature measurements were done using copper/constantan thermocouple junctions. A steady-state temperature was maintained for a period of 30 min. Temperature was measured every 4--10 s in the absence of the radiofrequency field. At the end of the treatment, continuous temperature samplings were recorded until the measured temperatures returned to base-line levels. The periphery of each treated site was delineated to aid in identifying the site at the time of biopsy. All animals recovered without incident. Antibiotics were administered i.m. for prophylaxis.

Tissue Analysis. Untreated and treated sites were sampled 28 days after initiation of hyperthermia treatment. Sections from the treated sites included the entire parenchyma and two capsular surfaces (see Chart 1C). Three samples, identified as proximal (P), middle (M), and distal (D), were obtained from each treated site, corresponding to the locations...
LIVER THERMOSENSITIVITY

Chart 1. A, anatomy of the canine liver (cephalad view). Left lateral lobe (A), left medial lobe (B), right medial lobe (C), right lateral lobe (D), and caudate process of caudate lobe (E). A pair of disk-shaped electrodes is shown placed against Lobe B. Site A was conditioned to 43.0°C/30 min and challenged at either 44.5°C/30 min or 47.5°C/30 min, 4 h later. Site E received a single 43.0°C/30-min treatment. Site C received a single treatment of either 44.5°C/30 min or 47.5°C/30 min. Site B was sham treated. Site D was not treated and controlled for anesthetic (halothane) injury. B, cross-sectional view of a liver lobe showing placement of a pair of direct-contact disk-shaped electrodes and definition of coordinate system. Electrode diameter was 3 cm. Electrodes were separated by distance d. Single point temperatures were measured at x, y, and z using thermocouple sensors. Temperature distributions were measured along the X, Y, and Z axes. C, side view of liver lobe illustrating areas of tissue sampling. Three 3-mm-thick slices were sectioned and prepared for light microscopy. Ultrathin sections were prepared for electron microscopy.

where temperature was measured during the treatment (Chart 1). Each sample included an area of untreated normal liver tissue as control. A total of 36 treated samples was obtained. A single sample was collected from each of the sham-treated and control sites, for an additional total of eight samples. Thick (3 mm) blocks were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned, and stained with hematoxylin-eosin and Gomori’s trichrome for light microscopy. Ultrathin sections from selected sites were collected within the first few min and immediately fixed in 2% glutaraldehyde solution in preparation for transmission EM.

All histological sections were coded, examined simultaneously (in a double-headed microscope), and graded by two investigators unaware of the corresponding treatment. If the two observers differed in assigning scores to the same lesion, an average value was recorded.

Twelve parameters of injury were used to quantify the magnitude of damage to each one of the following histological structures (categories): liver capsule; liver lobules; portal areas, and central veins (Table 1). The parameters graded in each of these structures were: (a) edema; (b) congestion; (c) hemorrhage; (d) cellular inflammatory exudate; (e) stagnation of erythrocytes in veins (as index of possible venous occlusion); (f) abnormal staining affinity of hepatocytes; (g) enlargement of and/or pigment accumulation in Kupffer cells; (h) necrosis; (i) cell loss (lack of cells independent of cell necrosis); (j) fibrosis; (k) portal-central fibrous bridging; and (l) distortion of lobular architecture (caused by fibrosis, cell loss, necrosis, or combinations). Several of these parameters are not applicable in one or more structures and are identified as such in Table 1.

After initial review of multiple representative ("Standard") sections, a scoring system of 5 grades (0 = normal; 1–4 = increasing degree of abnormality) was devised by labeling as 4 the most severe lesion in each parameter (for each applicable structure) and labeling as 1 the least severe one. Grades 2 and 3 were then established at evenly spaced intervals between 1 and 4; obviously lesions did not always fit within a preconceived grade and were then ascribed to the closest grade.

Some parameters (c, d, h, i, j) were graded by estimating the average representative linear dimension of the lesion in the plane of the section (two sections were evaluated from each block). Others (a, b, e, f, g, k, l) were graded by comparing the intensity and/or extent of the lesion with the predetermined standard sections.

Temperature Distributions. In a separate experiment we used a single animal to measure the radial and axial temperature distributions induced in the liver parenchyma. We selected Lobe A for this measurement because it was easily accessible. A single-junction thermocouple sensor was inserted through each of one of three sections of implanted Teflon tubing (18 gauge) oriented at 90 degrees to each other, as shown in Chart 1B. We measured the temperature along the X, Y, and Z axes, using the methodology described elsewhere (7).

Blood Flow Rate Measurements. A single experiment was performed in order to determine the effects of prolonged anesthesia on the effective rate of blood flowing through the liver parenchyma. The pair of parallel-plate disk-shaped electrodes was sequentially placed across liver Lobes

Table 1

<table>
<thead>
<tr>
<th>Item and Structure</th>
<th>Capsule</th>
<th>Lobules</th>
<th>Portal areas</th>
<th>Central veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Edema</td>
<td>0.175</td>
<td>0.071</td>
<td>0.00</td>
<td>−0.012</td>
</tr>
<tr>
<td>(b) Congestion</td>
<td>−0.063</td>
<td>0.018</td>
<td>0.147</td>
<td>−0.156</td>
</tr>
<tr>
<td>(c) Hemorrhage</td>
<td>0.340</td>
<td>0.060</td>
<td>0.197</td>
<td>0.144</td>
</tr>
<tr>
<td>(d) Cellular inflammatory exudate</td>
<td>0.078</td>
<td>−0.041</td>
<td>0.290</td>
<td>0.046</td>
</tr>
<tr>
<td>(e) Stagnation of RBCs in veins</td>
<td>NA*</td>
<td>NA</td>
<td>0.100</td>
<td>0.238</td>
</tr>
<tr>
<td>(f) Abnormal cell staining affinity</td>
<td>NA</td>
<td>0.104</td>
<td>0.002</td>
<td>NA</td>
</tr>
<tr>
<td>(g) Kupffer cell alterations</td>
<td>NA</td>
<td>0.00</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(h) Necrosis</td>
<td>0.00</td>
<td>0.079</td>
<td>0.00</td>
<td>0.093</td>
</tr>
<tr>
<td>(i) Cell loss</td>
<td>0.212</td>
<td>0.353</td>
<td>0.138</td>
<td></td>
</tr>
<tr>
<td>(j) Fibrosis</td>
<td>0.297</td>
<td>0.289</td>
<td>0.237</td>
<td>0.321</td>
</tr>
<tr>
<td>(k) Bridging</td>
<td>NA</td>
<td>NA</td>
<td>0.097</td>
<td>0.097</td>
</tr>
<tr>
<td>(l) Architectural distortion</td>
<td>NA</td>
<td>0.306</td>
<td>0.319</td>
<td>0.160</td>
</tr>
</tbody>
</table>

* The abbreviation used is EM, electron microscopy.

NA, not applicable.
A, B, and E (see Chart 1A), and a small temperature increment of 2°C was induced. Three-point continuous temperature measurements were recorded during the phase of temperature decay, until normothermia was reestablished. Similar measurements were performed at the same site 4 h later. A final measurement was taken after the animal’s sacrifice to obtain an estimate of the magnitude of thermal conductivity.

Data Analysis. A steady-state temperature was computed for each measurement point, at each treatment site (e.g., Site B, Points x, y, and z; Chart 1B) by averaging temperature measurements obtained at that point over a period of 30 min. The mean steady-state temperature was then computed and assigned to that site.

Pearson’s correlation coefficients were computed between the scores assigned to a given item, in a given category, and the steady-state temperatures assigned to the same item, in the same category. Data points originating from sites exposed to a single heat dose, control sites, and sham-treated sites were considered in this computation (total of 32 data points).

Average observed differences in the histopathological score observed in once- and twice-heated liver lobes and the statistical significance of these differences (significance probabilities of the treatment effect) were computed as follows. Average scores were computed for each item, in each category, by combining the scores of all three biopsy samples (P, M, and D of Chart 1C) originating from the same site. For a given animal, the average score of a given item, in a given category, observed in Site C (site of single heat treatment) was subtracted from the average score of the same item, in the same category, observed in Site A (site of double heat treatment). These differences were averaged over the total number of animals (three degrees of freedom), and a t test of statistical significance was performed (8). A value of $P \leq 0.05$ was considered statistically significant.

The percentage of maximal damage in a given category was computed by adding the scores of the “applicable parameters,” and then dividing by the maximum score possible in that same category (normalization). For example, the percentage of maximal damage to the liver lobules was computed by adding the scores for cell loss, fibrosis, and architectural distortion (Items I, J, and K), and then dividing by 12. Total liver damage was computed as the normalized sum of the percentage of damage seen in the four categories. Applicable parameters were considered those that exhibited a temperature correlation coefficient $r \geq 0.20$. This cutoff value was arbitrarily selected and is not related in any way to the level of statistical significance of these temperature correlation coefficients.

Effective blood flow rates were estimated from the temperature washout data by solving the bioheat equation with certain approximations (9). Heat loss was assumed to occur only by the process of convection. Arterial temperature was assumed to be equal to systemic temperature.

RESULTS

None of the animals showed any evidence of systemic ill effects attributable to hyperthermia. At the time of biopsy, several areas of adhesion between liver lobes and between treated lobes and the abdominal wall were seen. Several treated areas, especially the ones exposed to high temperatures, showed marked discoloration and appeared to be firmer than the rest of the parenchyma.

EM of control sites showed no evidence of cellular damage attributable to anesthetic injury. EM samples of treated areas demonstrated the same types of lesions seen by light microscopy. A dose-response relationship could not be demonstrated from these EM observations.

The light microscopic study and grading showed, as expected, multiple, severe lesions of the capsule (edema, hemorrhage, fibrosis, etc.). These were, to a great extent, the result of surgery (as can be observed in the controls of Chart 3A). Thermal injury was observed mainly in the lobules and to a lesser degree in portal areas and central veins. Congestion and edema were usually not severe and correlated poorly with temperature. The inflammatory exudate was usually lymphohistiocytic, moderate, and limited to the portal areas. Neither prominence of Kupffer cells nor variation in the staining affinity of hepatocytes was consistent or severe. No definite venoocclusive lesion was found in either central or portal veins, but stagnation of RBC was noted in a few central veins.

The significant changes were: large, confluent areas of coagulation necrosis involving entire lobules, portal areas, and central veins with minimal, peripheral granulocytic infiltrate; focal fibrosis in lobules, portal areas, and central veins, or discrete large scars adjacent to areas of necrosis or to the capsule (portal-central fibrous bridging was uncommon); and cell loss (different from active cell necrosis) observed mainly in the lobules as interruptions in the cell plates. As the result of the above, distortion of the lobular architecture often occurred.

Table 1 displays the correlation coefficients ($r$) of each parameter, in each category, with temperature. Most of these coefficients are positive; those that are negative are within the noise level. We consider relatively good correlations only the ones that have $r \geq 0.20$. From the data in Table 1, we conclude that the items that correlated best with temperature were: cell loss in the liver lobules and portal areas; fibrosis in all four categories; and architectural alterations in the liver lobules and the portal areas. Hemorrhage in the capsule, inflammatory exudate in the portal areas, and stagnation of RBC in the central veins also exhibited relatively good correlations with temperature.

A summary of the average observed differences in histopathological score observed in once- and twice-heated liver lobes is shown in Table 2. Each entry in the table represents the difference between the damage score assigned to the site that was exposed to a single heat treatment and the score assigned to the site that was treated twice (“thermotolerant” site). A negative value indicates that the liver lobe that received the single heat treatment suffered more damage than the liver lobe that received two heat treatments (“thermotolerance”). A positive value indicates that the lobe that received two treatments was damaged more than the liver lobe that received the single treatment. The statistical level of significance of these differences (significance probabilities of the treatment effect) is coded. Most of the entries in the table are nonsignificant. The only entry that is significant at the $P \leq 0.05$ level is the magnitude of hemorrhage in the liver lobules. Seven additional entries are marginally significant (edema and fibrosis in the liver capsule; fibrosis and architecture in the lobules; and edema, fibrosis, and architecture in the central veins).

Chart 2 displays families of temperature distributions measured along the $X$, $Y$, and $Z$ axes. Temperature distributions within the heated cylinder are reasonably uniform. The characteristic “rabbit ears” associated with parallel-plate radiofrequency heating become more pronounced at the high power densities. Temperature distributions along the $Y$ axis converge towards the systemic body temperature at distances greater than 3.5 cm into the liver parenchyma. Temperature distributions are skewed towards the distal part of the liver lobe, possibly demonstrating the effect of preheated blood flowing through the heated volume.

Chart 3 displays percentages of maximal tissue injury when assessed from the damage seen in the liver capsule, liver lobules,
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portal areas, and central veins, respectively. Chart 4 displays the integrated liver damage as a function of temperature. Sham-treated and control data are also displayed in Charts 3 and 4. In both of these, it is apparent that once temperatures above baseline are achieved, the degree of damage is temperature independent (lack of a monotonically increasing dose-response relationship). In fact, for several of the end points (e.g., damage to liver lobules and portal areas), there appears to be slight protection at higher temperatures rather than increased damage at higher temperatures. Single 30-min heat exposures at or above 41°C induced significantly greater levels of liver damage than those observed in the sham-treated and control groups (Chart 4). This indicates that the liver is a highly thermosensitive organ. A single 30-min heat exposure to 45°C induced a level of liver damage of approximately 22% (Chart 4). A double heat exposure of 43.0°C/30 min followed by 45.4°C/30 min, 4 h later, induced a level of liver damage of approximately 44% (Chart 4). Comparison at these two points indicates that there is a significantly greater level of damage in the double exposure group.

Percentages of tissue injury were also plotted against power density (data not shown). The power density required to maintain a given temperature during the second treatment was shown to be much less than the power density required to maintain the same, or even lower, temperature during the first treatment. Effective blood flow rates were computed from the temperature wash-out data at the end of the first and the second treatments. A scatterplot of these data is shown in Chart 5. The 45-degree line would indicate no changes in measured effective blood flow rates. This chart illustrates a significant reduction of the effective blood flow rate at the end of the second treatment. Chart 6 shows the time variation of a series of effective blood flow measurements performed over a period of 7 h. Although there is some point-to-point variation, these data are inconsistent with the hypothesis of a systemic effect, such as reduced cardiac output.

DISCUSSION

Induction of localized hyperthermia in deep-seated tumors involves the use of electromagnetic or ultrasonic energy supplied by one or more applicators. For noninvasive heating, the wave nature of these modalities necessitates the presence of high flux densities and possibly high specific absorption rates which could potentially lead to unacceptable temperatures in more superficial layers of normal tissue (e.g., skin, adipose tissue, and skeletal muscle). The use of external cooling can certainly protect the skin and partially the fat layer, but in general all three types of tissue are potentially at risk. In addition, induction of regional hyperthermia using electromagnetic energy could produce local-

Table 2

<table>
<thead>
<tr>
<th>Item and structure</th>
<th>Capsule</th>
<th>Lobules</th>
<th>Portal areas</th>
<th>Central veins</th>
</tr>
</thead>
<tbody>
<tr>
<td>(a) Edema</td>
<td>0.83b</td>
<td>-0.08b</td>
<td>0.08b</td>
<td>-0.16b</td>
</tr>
<tr>
<td>(b) Congestion</td>
<td>-0.06c</td>
<td>0.75b</td>
<td>-0.41b</td>
<td>0.00b</td>
</tr>
<tr>
<td>(c) Hemorrhage</td>
<td>0.16c</td>
<td>0.91d</td>
<td>-0.33b</td>
<td>0.06d</td>
</tr>
<tr>
<td>(d) Cellular inflammatory exudate</td>
<td>0.58b</td>
<td>-0.08b</td>
<td>0.16b</td>
<td>-0.08b</td>
</tr>
<tr>
<td>(e) Stagnation of RBCs in veins</td>
<td>NA</td>
<td>NA</td>
<td>0.00c</td>
<td>0.33c</td>
</tr>
<tr>
<td>(f) Abnormal cell staining affinity</td>
<td>NA</td>
<td>-0.08c</td>
<td>-0.25b</td>
<td>NA</td>
</tr>
<tr>
<td>(g) Kupffer cell alterations</td>
<td>NA</td>
<td>-0.25c</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>(h) Necrosis</td>
<td>0.00c</td>
<td>1.50b</td>
<td>0.75b</td>
<td>0.75b</td>
</tr>
<tr>
<td>(i) Cell loss</td>
<td>NA</td>
<td>0.91b</td>
<td>0.49b</td>
<td>0.50b</td>
</tr>
<tr>
<td>(j) Fibrosis</td>
<td>0.66d</td>
<td>1.58d</td>
<td>0.75b</td>
<td>1.16d</td>
</tr>
<tr>
<td>(k) Bridging</td>
<td>NA</td>
<td>NA</td>
<td>0.58b</td>
<td>0.58b</td>
</tr>
<tr>
<td>(l) Architectural distortion</td>
<td>NA</td>
<td>1.25a</td>
<td>1.25b</td>
<td>1.41a</td>
</tr>
</tbody>
</table>

* Significance (P) of treatment effect, 0.05 < P ≤ 0.10.
  b Significance, 0.10 < P ≤ 0.25.
  c Significance, 0.25 < P ≤ 0.50.
  d Significance, 0.02 < P ≤ 0.05.
  NA, not applicable.
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Chart 3. Percentage of maximal damage to liver capsule (A), liver lobules (B), portal areas (C), and central veins (D) versus temperature. Three data points (P, M, and I) have been averaged. Single-point temperature accuracy was ± 0.1°C. Steady-state temperatures were maintained for a period of 30 min. Estimated error in damage score was ± 10%. Bars, SE. O, single dose group; •, double dose group; □, sham-treated group; X, control group.

Chart 4. Percentage of maximal damage to canine liver versus temperature. Total liver damage was computed from the sum of the damage to liver capsule, liver lobules, portal areas, and central veins. For definition of symbols and other details, see legend for Chart 3.

Chart 5. Abscissa, effective blood flow rates computed from temperature washout data measured at the end of a single 43.0°C/30-min heat treatment; ordinate, effective blood flow rates computed from temperature wash-out data measured at the end of a second heat treatment of either 44.5°C/30 min or 47.5°C/30 min, given at the same site 4 h later. Blood flow rate expressed in units [ml/100 g/min]. Bars, SD. No effect represented by solid line at 45 degrees.

LIVER DAMAGE

EFFECTIVE BLOOD FLOW RATE

EFFECTIVE BLOOD FLOW RATE

'Hot spots' in any deep-seated normal tissue or organ that are not as well vascularized as the surrounding tissues, even if the magnitude of the electric field were the same throughout the treated volume. It is clear that the thermal sensitivity of various normal tissues and organs must be determined. In addition, some means must be devised to protect normal organs and tissues from the cytotoxic effects of elevated temperatures.

The thermal sensitivity of various rodent tissues and the effects of induction of thermotolerance in these tissues have been studied by many investigators. Law et al. (10) studied the heat...
tolerance and heat resistance of the mouse ear. Rice et al. (11), Suit and Blitzer (12), and Urano et al. (13) have studied the effect on the mouse foot. Hume and Marigold (14) investigated the heat response of the mouse small intestine.

The liver appears to be at risk for significant injury from localized or systemic hyperthermia (15–19). Lesions are consistently found, but their ultimate clinical effect may not be as important as expected (15). In the present study, chronic liver damage was quantified using histopathological criteria. The magnitude of liver injury in the acute phase (≤24 h) was not studied, although early light and electron microscopy might identify reliable predictors of long-term damage (15, 19). Standard biochemical liver function tests could not be used in this study since, in a given animal, each liver lobe received a different treatment.

Electron microscopy could better document the earlier stages of the observed lesions. Wills et al. (19) have used EM to study biopsies of human liver obtained before and within 2 days after 40°C–42°C/C/65–495 min. Evidence of ultrastructural damage in some hepatocytes was observed immediately after therapy (when no histological lesions were evident) and became progressively worse. By 2 days, there were numerous autophagic vacuoles, dilatation of Golgi cisternae, massive distention of rough endoplasmic reticulum, flocculent deposits in mitochondria, dilatation of bile canaliculi with loss of villi, and increase in bile granules. Most prominent were large vacuoles (as much as 12 μm in diameter) (19). Presumably these alterations result in necrosis of the damaged cells and extrusion from their plates in less than 4 wk (15).

The important findings of this study were (a) evidence of high thermosensitivity of liver tissue and (b) lack of clear evidence of thermotolerance induction. The protective effect of thermotolerance cannot be demonstrated in the present study. Did thermotolerance actually occur? With as much variability in the data as reported here, this is a difficult question to answer. Two reasons make us think it did not. (a) The damage values from sites treated twice, taken as a single group, are significantly higher than the damage values from sites treated once, also taken as a single group (Wilcoxon two-sample rank sum test; m = 8, n = 4, W = 39 where m is the number of observations in the large sample size, single dose group; n is the number of observations in the small sample size, double dose group; and W is the sum of the ranks obtained by the sample of size n.). (b) At the high temperature end (approximately 45°C), we would have expected reduced damage following two treatments if thermotolerance had been induced. In fact the opposite was seen.

Landry et al. (20) have shown that hepatic epithelial cells and freshly isolated hepatocytes from rat liver can become thermotolerant when conditioned with a 30-min heat treatment at 43°C. The effect reaches a full amplitude after a 4–8 h period at normothermic temperatures and lasts for more than 1 day at a level corresponding to a 50-fold increase in cellular thermotolerance (20). In addition, heat shock proteins are induced in the liver (20, 21). Appearance of such proteins has been correlated with development of thermotolerance.

Many possible reasons could account for our failure to demonstrate thermotolerance. Among the various possibilities are the following: different kinetics of thermotolerance induction in the intact liver; overshadowing vascular effects; lack of sensitivity of the assay; temporal and spatial sampling errors; and three-dimensional temperature nonuniformities. All of these are testable hypotheses. The hypothesis that the kinetics of thermotolerance induction is different in the canine liver is tenable. Among the remaining possibilities, the possibility that the protective effect of thermotolerance was overshadowed by overriding vascular effects is quite reasonable. The results of Chart 5 suggest that the effective rate of blood flowing through the treated liver parenchyma was reduced. It is not clear if this reduction is the result of vascular (or other) effects caused from the first (single) treatment or the result of the combined effects of the first and the second treatments. A reduction in blood flow rate could lead to a decrease in extracellular pH and a concurrent decrease in the amounts of nutrients available to liver cells. Both effects could render liver cells more susceptible to subsequent heat challenges and thereby overcome any protection afforded by thermotolerance.

Skiba and Collins (22, 23) have developed an in situ rat liver perfusion system. They have used this system to study a variety of hepatic biosynthetic functions at hyperthermic temperatures (24–26) in the presence or absence of chemotherapeutic agents (27, 28). Recently they have extended this technique to the canine liver (29). Blood flow rate through the liver parenchyma and the extracellular concentrations of various nutrients, such as glucose, lactate, and pyruvate, play an important role not only on the functional integrity of the organ but on the survival of the whole organism as well.

In these experiments, halothane has been used to maintain dogs under systemic anesthesia. Halothane has been shown to cause acute injury to normal liver at normal body temperatures (30–33). It is not known if the hepatotoxic effect of halothane is potentiated at elevated temperatures. Although we controlled for the effects of halothane in this study, a different anesthetic (e.g., nitrous oxide) might be used in a subsequent study in order to assess the influence of the anesthetic on the intrinsic thermal
sensitivity of the liver and/or the kinetic expression of thermotolerance.

Conclusions. An evaluation scheme, based on histopathological criteria, has been developed to quantify the magnitude of delayed heat damage in the liver. Among 12 parameters of tissue criteria, has been developed to quantify the magnitude of sensivity of the liver and/or the kinetic expression of thermo-
dence of hepatocyte loss; focal fibrosis; and distortion of lobular
injury, the ones that correlated best with heat dose were: evi-
strated in these experiments. It is likely that the protective effect
treatments. Thermotolerance induction was not clearly demon-
The liver appears to be quite sensitive to therapeutic heat
treatments. Thermotolerance induction was not clearly demon-
stration of data analysis.

ACKNOWLEDGMENTS

We wish to express our thanks to Dr. Byron W. Brown, Jr., for his critical review
of the process of data analysis.

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