PREDICTION OF SURVIVAL TIME OF RATS IN HOT ENVIRONMENTS[1]

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Abstract. A total of 136 male rats were exposed to terminal heat stress and the colonic heating curves were analyzed to determine equilibrium temperature, rate of heating from resting temperature to the equilibrium temperature, the difference between resting temperature and equilibrium temperature, and the rate of colonic temperature change during the first 30 minutes following attainment of the equilibrium temperature for formulation of an equation for prediction of survival time. It was determined by stepwise multiple linear regression that equilibrium temperature and rate of colonic temperature change were significantly correlated with survival time. A prediction equation was computed for control animals (N = 66) and applied to rats of different body weight (age), thermal exposure environment, and peripheral vascular tone (spontaneously hypertensive rats). The results indicated that the relationship between survival time and heating curve parameters was similar between different age groups with a mean error of 0.2% and 7.3% noted in predicted values for 10 and 7-week old animals, respectively. The predictive accuracy of the equation was, however, markedly reduced in control animals (36.2% error) and hypertensive animals (15.2% error) exposed to a less severe thermal environment.

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Survival time during terminal heating is generally regarded as the best indicator of heat resistance since it incorporates both the active and passive elements of thermoregulation. The magnitude of variability of thermal tolerance observed among individuals and species, as well as the terminal nature of the methodology, has limited the use of this measurement in the comparison of groups of animals suspected of altered thermoregulatory capacity. In a recent report, Ohara and co-workers (1975) have indicated that the survival time of rats subjected to a standardized, acute heat stress could be accurately predicted (measured survival time − predicted survival time/measured survival time = 1.8 ± 17.7%) on the basis of parameters obtained from the rectal temperature curve prior to observation of gross heat damage, thus, enabling repeated testing of individuals. They also found that the survival time predicted for short-term heat exposures was significantly correlated with the measured survival times of animals in chronic heat and suggested that chronic heat tolerance might be estimated from acute heat stress studies. Their prediction equation was derived from data obtained from animals exposed to a single environment (42.5°C, 40% relative humidity) with predictive accuracy tested in only 5 animals. Furthermore, Ohara *et al* (1975) showed that the prediction equation differed between male and female rats, suggesting that other physical or physiological factors may influence the relationship between survival time and heating curve parameters.

In view of the potential importance of the survival time prediction equation for evaluating differences in thermal resistance following activities such as acclimation, drug usage or toxic substance exposure, we have conducted a series of experiments to determine its general applicability to groups of animals differing in weight (age), thermal stress exposure conditions and systemic vascular tone (hypertension).

MATERIALS AND METHODS

A total of 136 male rats were grouped as

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TABLE 1

Group statistics of animals exposed to terminal heat stress.

Group	Age Weeks	No. of Animals Exposed	Chamber Air Temperature	Body Weight (g)	No. of Animals Analyzed**
I	10	66	39.5 C	329.4± 1.8	61
II	7	28	39.5 C	182.2± 4.5	27
III	13	13	37.0 C	366.0±10.0	13
IV*	14–25	29	37.0 C	312.1± 9.5	22

*Okamoto-Aoki strain, spontaneously hypertensive rats. BP=176±3 mm Hg. All other animals were of the Sprague-Dawley strain.

**A portion of animals examined did not demonstrate an equilibrium temperature and were not included in the analysis.

shown in table 1. The animals were housed individually or 2 per cage at 23±2°C with a 14-hour light and 10-hour dark photoperiod. Food and water were freely available prior to heat exposure. The mean blood pressure of SH rats was determined under light seconal anesthesia (0.06 ml/100g) with a Friedman:Freed microphonic manometer with indirect tail cuff.

The heating apparatus consisted of 170 liter (Groups III, IV) and 480 liter (Groups I, II) cabinets maintained at 37.0±0.5°C, 51±6% relative humidity (rh) and 39.5±0.9°C, 28±4% rh, respectively. Colonic temperature and air temperature in the chamber were monitored to the nearest 0.1°C with thermistor probes and recorded on a Varian Model A-25 recorder. The thermistor probe was inserted 5–6 cm through the rectum and taped to the tail. The animals were returned to 24 liter holding cages for 30 minutes and the resting colonic temperature noted. The rats were then placed in the heat chamber and colonic temperature was recorded until cessation of respiratory movements. The heating curves obtained were fitted by eye and the equilibrium temperature (Te), the increase in °C from the resting temperature to the equilibrium temperature (ΔTe), the rate of colonic temperature elevation in °C/hour from the resting temperature to the equilibrium temperature (Gt_1), and the rate of change in colonic temperature over the 30 minute time period following attainment of the equilibrium temperature (Gt_2) were determined after the method of Ohara *et al* (1975).

The data were initially applied to the survival time prediction equation arrived at by Ohara and co-workers for male rats heated at 42.5° and 40% rh and the differences between measured and predicted values were compared by paired t-tests. Subsequently, a prediction equation was derived for the data of Groups I and II by stepwise multiple linear regression analysis so that the predictive significance of each parameter individually and in combination with other parameters was determined. The predictive accuracy of the equation was then determined for the data of 37°C exposed control and hypertensive animals of Groups III and IV.

RESULTS

The colonic heating curve patterns were highly variable among individuals, ranging from a single stage linear increase from the resting to the lethal temperature (10% of animals examined) to the 3-stage curve described by Ohara *et al* (1975). The survival values for the study as a whole ranged from 56.8 to 420.0 minutes with a mean of 171.2±78.0 minutes.

The equation derived by Ohara *et al* (1975) for male rats exposed to ambient temperature 42.5°C ($\log S = 8.6458 - 4.2437 (\log Te) - 0.0060 (\log \Delta Te) + 0.3734 \times 10^{-Gt_2}$) did not accurately predict the survival times of groups of animals of this study with mean errors of 10% to 50% noted in the predicted values among the different groups (table 2). The multiple regression analysis indicated that of the heating curve parameters examined, only Te and Gt_2 were significantly correlated with Ts. Figure 1 shows the relationship between Te or Gt_2 and Ts for 10 and 7-week old rats. The older animals (Group I) showed a high level of correlation of both Te and Gt_2 values with Ts ($P<0.001$). In younger animals (Group II) the Te was not highly correlated with Ts.

The regression analysis did indicate, however, that Te contributed signifiicantly to the precision of the prediction equation when examined in combination with Gt_2 values. Tests for equality of slopes and comparison of the regression line intercepts (Sokol and Rohlf 1969) indicated the relationships between Te,

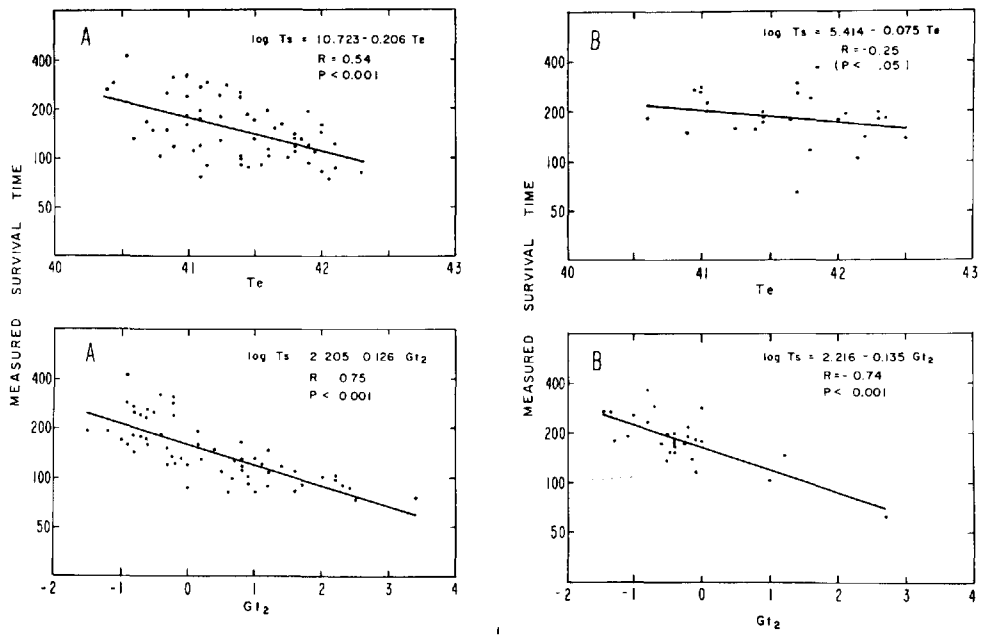

FIGURE 1. The relationship of equilibrium temperature (Te) and the rate of heating during the first 30 minutes following the attainment of the equilibrium temperature (Gt_2) with survival time. Graphs A and B show Group I and II data, respectively. The equations in the right corner of each figure were derived from that specific set of data. The solid lines were obtained from the equation derived for the pooled data of Groups I and II.

Gt_2 and Ts were not significantly different between the 2 groups. Consequently, the data were pooled for the derivation of the prediction equation:

$$\log Ts = 8.2193 + 0.1313\ Gt_2 - 0.1449\ Te$$

(1) where $R = 0.869$

$P < 0.001$

The scatter of points and a comparison of the equation lines of the 10 and 7-week old animals with the pooled equation (1) line is shown in figure 2. In this instance, Ts was plotted as a function of Gt_2 and the Te deviation from the mean value was expressed as an adjusted Gt_2 value derived from equation (1).

$$(2)\ \log Ts = bo + b_1\ Gt_2 + b_2\ Te$$
$$= bo + b_2\ \overline{Te} + b_1\ Gt_2 + b_2\ Te - b_2\ \overline{Te}$$
$$= (bo + b_2\ \overline{Te}) + b_1\ (Gt_2 + b_2/b_1\ (Te - \overline{Te}))$$

$$(3)\ (Tt_2)^1 = (Gt_2 + b_2/b_1\ (Te - \overline{Te}))$$

where $(Gt_2)^1$ = adjusted Gt_2

b_1, b_2 = regression coefficients of Gt_2 and Te, respectively

$\overline{Te}$ = sample population mean Te value

Te = equilibrium temperature

The combination of Gt_2 and Te values markedly improved the correlation between measured survival time and heating curve parameters, as compared to their usage as individual parameters in Group I. The correlation coefficient obtained for combined Gt_2-Te values and Ts was, however, not increased above that observed for Gt_2 values used singly in Group 2. There were no significant differences between the 2 groups in the

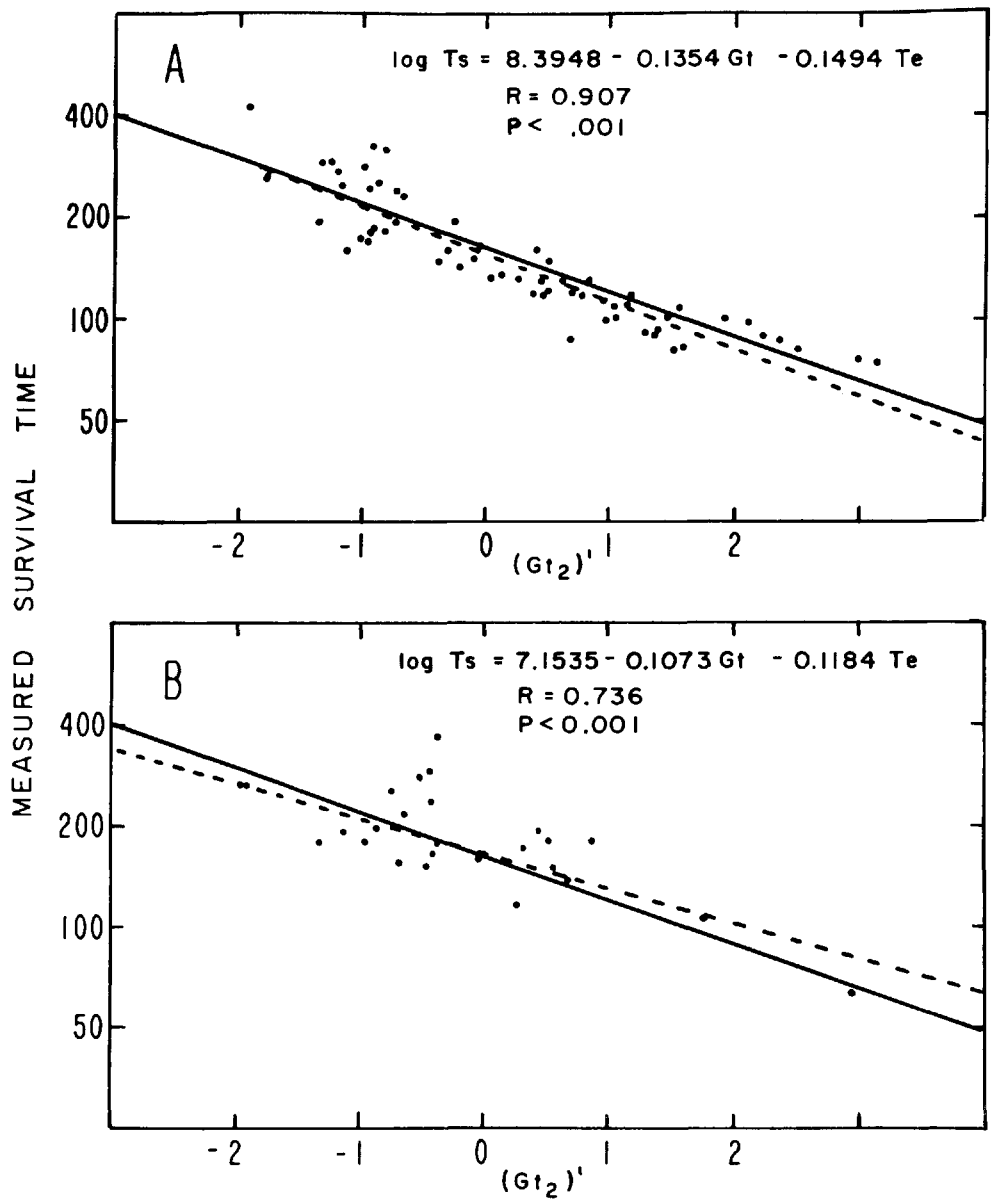

FIGURE 2. The relationship of the adjusted Gt_2 (see text) with survival time for Group I (A) and Group II (B). The equations in the right corner of each figure were derived for that specific set of data. The solid lines were obtained from the equation derived from the pooled data of Groups I and II whereas the dashed lines were obtained from the equations derived for the individual groups.

slopes or intercepts of the equation lines obtained from the equations derived for each group, nor between the equation line of each group and that obtained from equation (1), derived from the pooled data of Groups I and II.

The predicted survival times of 37°C exposed normotensive and hypertensive rats, as calculated from equation (1), were significantly lower than measured survival times (table 2). There was a distinct grouping of the regression plots of actual *vs.* predicted survival time according to the thermal exposure conditions

TABLE 2

The measured and predicted survival times calculated from the equation of this study and that of Ohara et al *(1975).*

Group	Measured Ts	Ohara *et al*	Predicted % Difference	Present Study	% Difference
I	159.0± 9.4	143.1±18.3	10.0*	159.3± 7.1	0.2
II	191.5±11.8	168.3± 8.9	12.1*	177.4±10.0	7.3
III	251.0±25.1▲	120.8± 6.7	51.8*	160.0±11.3	36.2*
IV	129.7±11.6●	100.3± 3.0●	22.6*	109.9± 8.1●	15.2*

The data are presented as mean±SEM. A triangle (▲) or closed circle (●) indicates a significant difference ($P<.05$ or greater) from Groups I and III, respectively. An asterisk (*) indicates significant differences between measured and predicted survival times.

(fig. 3) indicating that the severity of the heating environment altered the relationship between heating curve parameters and survival time.

DISCUSSION

The thermal resistance of an animal reflects the integration of a number of activities which may be broadly categorized as cardiovascular, secretory or behavioral in nature. Cutaneous vasodilation is an important mechanism of dry heat loss in environments where the thermal gradient favors heat dissipation from the animal (Rand *et al* 1965). It greatly facilitates heat loss by surface evaporative cooling under conditions in which the thermal gradient allows limited heat flow from the organism or in which body temperature is lower than its surroundings. The primary method of heat loss of the rat in environments with air temperature exceeding core temperature, however, is the salivary gland secretion of fluid for evaporative cooling (Rodland and Hainsworth 1974; Stricker and Hainsworth 1971). Stricker and Hainsworth (1971) have shown that the removal of the salivary gland greatly reduced thermoregulatory effectiveness and resulted in a rapid elevation of body temperature with decreased ability to establish and maintain an equilibrium temperature. The effect of behavior on rodent thermoregulation has not been thoroughly investigated, but it is reasonable to assume that variation in posturing and escape activity may contribute to differences in thermal resistance observed among individuals. For each individual, the body temperature response to high ambient temperature reflects the interaction of these systems and in view of the numerous factors which may influence this response, the wide range of survival times encountered in the present and other studies (Adolph 1947; Ohara *et al* 1975) is not surprising. This creates the problem, however, that the individual variability of response may mask differences in the measured thermal resistance of groups of animals in studies to show alterations in thermoregulatory ability. The development of a prediction equation which utilizes parameters from the heating curve prior to thermal damage could significantly increase the efficiency and usefulness of animal studies of thermal resistance through the repeated testing of individuals which then may serve as their own control.

Our findings indicate that the survival time prediction equation provided by Ohara *et al* (1975) did not accurately describe the relationship of body temperature response and survival time of our data. In each of the groups examined, the mean predicted survival time was significantly lower than the measured survival time (table 2). The degree of error appeared to be inversely related to the severity of the heat stress imposed on the animals, suggesting that the differences were due to the less severe heating conditions of this study. The significant reduction in the predicted survival time of 37°C, 40% rh exposed animals, obtained through the use of prediction equation (1) which was derived from data of 39.5°C, 28% rh exposed rats, further indicates that the methodology of heating may have a marked effect on the predic-

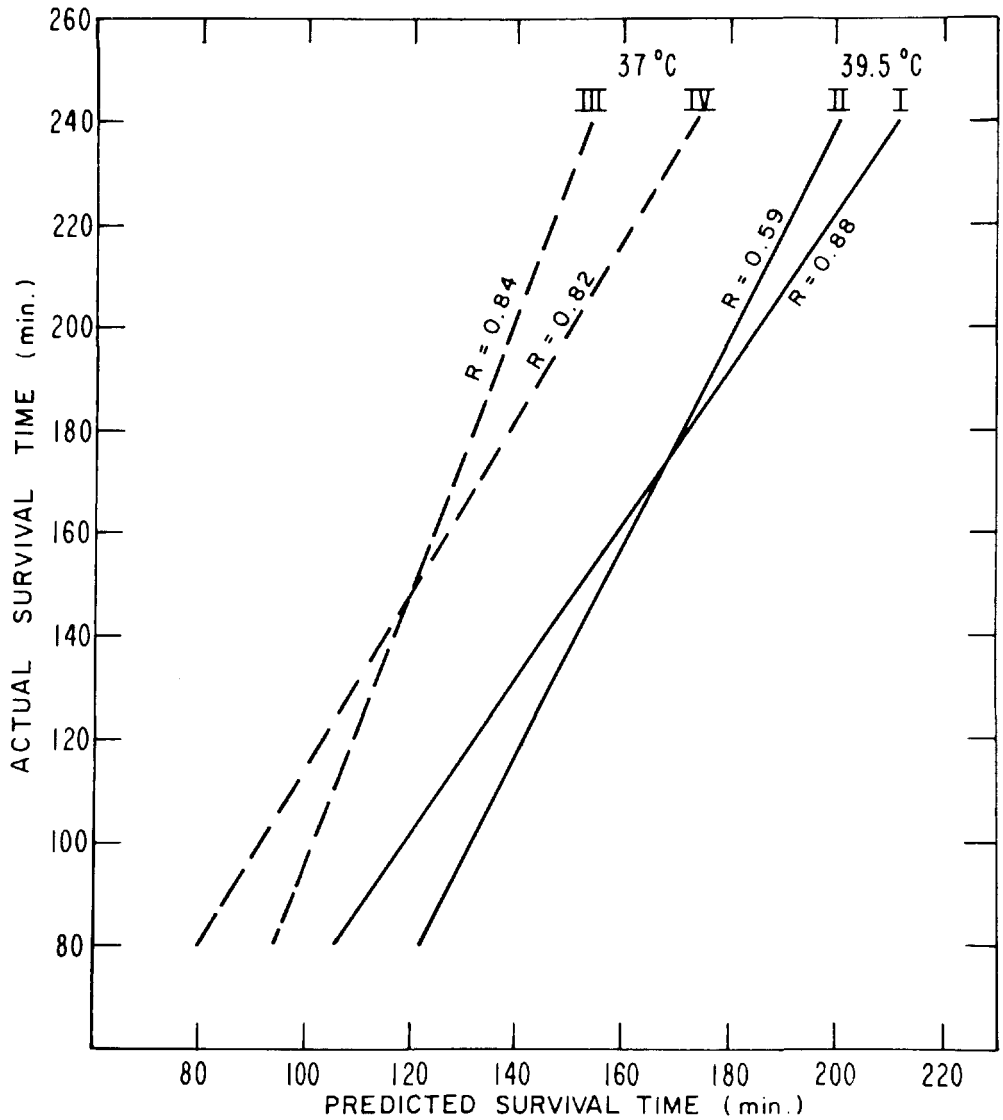

FIGURE 3. Linear regression plots of measured and predicted survival times. Predicted values were computed from the equation obtained for 39.5°C exposed animals for each of the groups examined. I=10 week old; II=7 week old; III=13 week old; IV=14–25 week old spontaneously hypertensive rats (SHR).

tive accuracy of the equation obtained. This in itself is not a major problem since the testing procedures and environmental exposure conditions can be standardized to allow comparisons between groups of animals. The similarity of SHR and control (37°C) regression plots of actual *vs.* predicted survival times (fig. 3) indicates that an equation may be derived that is generally applicable to groups of similarly exposed animals possessing variable levels of thermoregulatory effectiveness. It should be emphasized that while the nature of the equation derived by regression analysis is such that the mean predicted Ts value will be essentially the same as the measured Ts value for the group of data from which the equation was obtained, this does not necessarily mean that for individual data points the

equation values will faithfully follow measured values with homogeneous error throughout the range of thermal tolerance observed. The scatter of Group I data points about the ideal line with measured plotted against predicted survival time (fig. 4) makes it apparent that the equation is reliable for survival times below approximately 200 minutes but is less accurate at higher values.

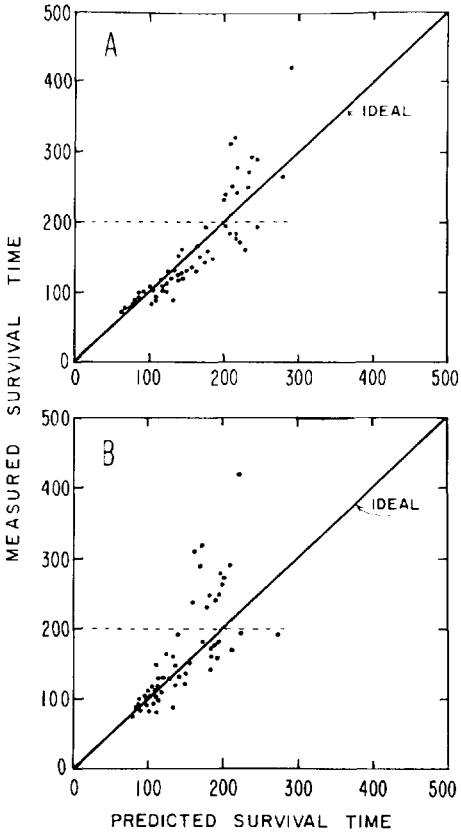

FIGURE 4. The pattern of Group I data point scatter about the ideal line with the measured plotted against the predicted survival time. Graph A shows predicted values computed from equation (1) of this study and Graph B from the equation of Ohara *et al* (1975).

Even with the development of very accurate prediction equations, the use of this tool may be limited. It is mandatory that an animal demonstrate a Te before any of the equations which have been discussed can be applied. Our data show that 10% of the animals exposed to 39.5° and 37°C exhibit linear or marginally linear heating patterns and thus do not meet this requirement and must be eliminated from the analysis (table 1). Furthermore, it is difficult to determine at what body temperature the heating must be terminated to prevent irreversible damage. Buchsbaum *et al* (1970) have reported membrane and cellular disintegration in rodent tissues maintained at approximately 42°C and Brauer *et al* (1963) observed biochemical alterations at 41.5°C. In view of these findings, it is probably resonable to assume that core temperatures should not exceed 41.5°C in animals which are to be reused. At a rectal temperature of 41.5°C, a significant percentage of the animals might still be expected to incur some degree of thermal damage, and the effect of even slight thermally-induced alterations on the subsequent response to secondary experimental treatments such as drug or toxic substance administration would have to be determined. A close inspection of the data and prediction equations obtained in this study and by Ohara and co-workers (1975) reveals that the slope of the heating curve following attainment of the equilibrium temperature provides the single most important indication of the overall thermal resistance of the animal. In the present study, we defined the Gt_2 as the curve slope measured at 30 minutes since it was felt that this time interval was sufficient for Gt_2 determination without undue exposure of the animal to elevated core temperature post attaintment of the Te. However, in studies in which the animal would be removed from heat stress at a core temperature of 41.5°C an additional portion of the sample population would be eliminated from the analysis. The average Te for this study was $41.4° \pm 0.5$°C and a total of 43% of the animals examined exhibited a Te equal to or greater than 41.5°C. Thus, a strict adherence to sampling termination at core temperature 41.5°C or at even slightly higher temperatures would result in the loss of ability to determine Gt_2 in many animals exhibiting positive Gt_2 values.

If the investigator feels he may validly sustain the loss of the portion of animals which do not demonstrate a Te and allow the heating of animals beyond 41.5°C so

that all individuals capable of attaining an equilibrium temperature will be included in the analysis, the use of the prediction equation may provide a tool for comparing pre- and post-treatment thermoregulatory ability within a group of animals. We do not feel, however, that the precision of the equation at higher survival values (Ts > 200 min) warrants the use of the equation for individual animal evaluation. Further research is needed to determine if heat exposure at less severe environmental conditions would result in fewer numbers of animals being unable to establish an equilibrium temperature or a lowering of the mean equilibrium temperature. Even if these attempts were successful, however, the Ts would be lengthened considerably and we suspect that the predictive accuracy of the equation derived for such data would be compromised.

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