Evidence of injury by heat in mammalian tissues

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Evidence of injury by heat in mammalian tissues. Am. J. Physiol. 206(5): 1057-1061, 1964.—Some possible criteria of injury by heat were investigated using slices of rat liver, cerebral cortex, and renal cortex heated in vitro. The following were measured at approximately 1-C intervals between 40 and 47.5 C, and in each case the results were compared with controls incubated for the same time at 38 C: QO2, the increase of ammonia, urea, cholesterol, and acid phosphatase in the suspension medium, and the leakage of Rb186 from tissues labeled with this isotope in vivo. In general, deviation from the control at a given temperature increased with time. For a given tissue, several or all of these deviated from the control at about the same temperature. For cerebral cortex this was about 43 C and for liver about 45 C. The most sensitive indicator of injury appeared to be the accumulation of ammonia in the medium, and this deviated from controls at 40 C for renal cortex and 42 C for liver and cerebral cortex. Thus, there is evidence for several biochemical changes produced by heating in vitro to temperatures at or below those at which irreversible changes occur in vivo.

METHODS

Tissues from well-fed male albino rats weighing 450-550 g were used. The animals were decapitated and the organs to be used were removed rapidly and transferred to covered petri dishes kept on cracked ice. Tissue slices, approximately 0.5 mm thick, were prepared immediately in a cold room at 5 C. Liver was sliced with the instrument described by Martin (22). Cerebral cortex slices were obtained using a razor blade and Lucite template (11). Renal cortex was sliced with a Stadie microtome (28). The tissue slices were blotted on filter paper, rapidly weighed on a microtorsion balance, and transferred to chilled respirometer flasks. The suspension medium was Krebs' medium III (20); the gas phase was 100% oxygen. The vessels were placed at a given experimental temperature for a thermoequilibration period of 15 min. Following this, oxygen consumption was determined manometrically from readings taken every 10 min and chemical determinations were performed at the end of a given experimental period as described below.

The concentration of ammonia was determined in the suspension medium after cooling the contents of the vessel in cracked ice and centrifuging for 3 min at 1,500 g. The concentration of ammonia was measured by the microdiffusion borate-HCl method of Conway (8). Urea was determined in another aliquot of the same fluid by the method of Watt and Crisp (30). In other experiments, total cholesterol in the suspension medium was determined by a modification of the method of Abell and Chen (2) and intermolecular bonding may also be responsible for heat death (3).

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hyperthermia temperature tissue metabolism
Comparison of oxygen consumption of rat liver, cerebral cortex, and renal cortex slices at supranormal temperatures with oxygen consumption of these tissues at 38 C (control)

<table>
<thead>
<tr>
<th>Tissue</th>
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<th>No. of Animals</th>
<th>% Deviation From Control Time, min</th>
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<tr>
<td></td>
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</tbody>
</table>

Cerebral cortex

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<th>Temp., C</th>
<th>No. of Animals</th>
<th>% Deviation From Control Time, min</th>
</tr>
</thead>
<tbody>
<tr>
<td>38</td>
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<td>+19.5  +8.1 +7.0 +6.0 +3.8 +0</td>
</tr>
<tr>
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<tr>
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Renal cortex

<table>
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<th>Temp., C</th>
<th>No. of Animals</th>
<th>% Deviation From Control Time, min</th>
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<tr>
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<tr>
<td>43</td>
<td>3</td>
<td>+15.0 +13.0 +11.5 +8.5 +9.0 +4.8</td>
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</tbody>
</table>

Determined by the method of Zak (31). For the determination of acid phosphatase, tris buffer (0.1 M at pH 7.25) was substituted for an equal volume of phosphate buffer and the enzyme activity determined by estimating the amount of inorganic phosphate liberated from β-glycerolaldehyde phosphate according to the method of Berthet et al. (6).

In order to test the leakage of intracellular inorganic material, rats were placed on a potassium-deficient diet for 3 days and were then loaded with about 160 μC of Rb86Cl contained in 0.75 ml unlabeled 10% RbCl solution, injected subcutaneously. Tissues were removed after 24 hr and the accumulation of labeled rubidium was determined in the suspension medium after incubation at various temperatures for different lengths of time up to a maximum of 180 min. Rb86 was measured using a well-type scintillation detector.

In all instances the data are given as differences from the results obtained on aliquots of tissue from the same rat incubated for the same length of time at 38 C.

Results and Discussion

Oxygen consumption. A decrease in the rate of oxygen consumption (QO2) has been used by many investigators as an indicator of injury to tissues. When isolated tissues are incubated at supranormal temperatures, the QO2 first increases above that at 38 C and, if the temperature is sufficiently high, then decreases progressively with time (12). Table 1 gives data for three tissues at various temperatures and times of measurement. The initial increase in QO2, which occurs at all temperatures below 47.5 C, obscures the exact temperature at which damage, as indicated by a decrease in QO2, occurs. Nevertheless it is clear from the data that the QO2 of cerebral cortex slices falls progressively with time at 40 C, and that decrease below the rate at 38 C occurs after incubation for 1 hr at 42 C. These results agree roughly with those of Field et al. (12) who studied the reversibility of QO2 after exposure at supranormal temperatures. Liver, and especially renal cortex, are much less sensitive to damage by heat if the criterion is inhibition of oxygen consumption.

Additional experiments, not included in Table 1, were done to find whether evidence of damage at lower temperatures could be obtained by incubation for a longer time than 1 hr at 40, 41, and 42 C. However, at these temperatures the QO2 of liver slices was well maintained, and even at 42 C the QO2 after 180 minutes was only about 7% below that at 10 min and always higher than at 38 C. The QO2 of cerebral cortex slices fell progressively with time at all three temperatures. A decrease in QO2 below that found at 38 C required about 90-120 min at 40 and 41 C, and 40-50 min at 42 C.

Thus, cerebral cortex is more sensitive to damage to heat than are liver or renal cortex when the criterion of damage is a decrease in oxygen consumption. Damage occurs in cerebral cortex after about 60-90 min at 40 or 41 C. Damage to liver requires exposure at 45 C for about 1 hr.

Ammonia production. An increase in the production of ammonia by excised fish brain at supranormal temperatures with oxygen consumption of these tissues at 38 C (control).
HEAT INJURY

FIG. 1. Physiological and biochemical changes produced by heating rat cerebral cortex and liver slices in vitro. All data are expressed as differences from results obtained on aliquots of tissue from the same rat incubated for the same length of time at 38 °C. The columns are the means of 2-13 (usually 3-6) values.

Cholesterol accumulation. Fähraeus (9) demonstrated that when a fresh section of a tissue containing lipid particles was warmed and observed with a polarizing microscope, the cholesterol esters remain unaltered from 36.5 to 37 °C, but on further heating, the white flecks decreased in size, broke up into smaller pieces, and disappeared at about 43 °C. Recently, Lovelock (21) showed that the cholesterol content of human red blood corpuscles is considerably affected by temperature. Irvine et al. (16) found that the addition of cholesterol to basic diets of Pablum, or Pablum and pilchard oil, increased the resistance of goldfish to heat.

Determinations of cholesterol in the supernatant fluid were made after incubation of liver and cerebral cortex slices at 38, 40, 43, 45, and 47.5 °C. The results are included in Fig. 1. Although cholesterol increases in the medium with increase of temperature with both tissues, this does not appear to be as sensitive a criterion of injury as others studied.

Acid phosphatase. The opening of lysosomes and the consequent release of their internal enzymes plays an important role in the initiation of autolytic and necrotic phenomena which end in cell death (2). Heat changes the permeability of cell membranes for a variety of substances (3). The release of acid phosphatase as a result of rupture of lysosomes has been shown by several workers (25). The accumulation of this enzyme was therefore studied in the suspension medium of rat liver and cerebral cortex slices at different temperatures.

Very little acid phosphatase was found in the supernatant fluid from either liver or cerebral cortex at 38 and 42 °C. Higher temperatures were not studied because there was evidence of inactivation of the enzyme itself at 44 °C. Release of acid phosphatase thus appears to be an unsatisfactory indicator of damage from heat.

Leakage of Rb⁺⁻. Potassium is concentrated inside the cell against a concentration gradient, a process that requires energy. Disturbance of the energy yielding processes of the cell coupled to active transport of sodium or potassium could lead to leakage of potassium from the cell and ultimately to death of the cells. High temperatures do affect the potassium balance of animals. Kanter (18) found that the excretion of potassium in the dog is markedly elevated after exposure to high environmental temperatures, and high levels of potassium in the serum are found during fatal hyperthermia (13, 15). Benjamin et al. (4) found that heating rats at an environmental temperature of 42 °C until there were signs of imminent collapse produced a decrease in the potassium concentration of the brain. On the other hand, these workers (5)
reported that little release of potassium occurs from guinea pig brain exposed to 42°C in vitro for 90 min. In the present study, the effect of heat on the intracellular concentration was studied by labeling the intracellular phase with Rb⁶⁺ in vivo and then determining the leakage of this isotope into the suspension medium at various temperatures in vitro. Rb⁶⁺ was used because this isotope has a longer half-life than do readily available potassium isotopes. This substitution is justifiable since it is known that rubidium and potassium are similar physically and physiologically (26, 27, 29).

The results of these experiments are given in Table 3. Some leakage of Rb⁶⁺ occurred from control slices of both liver and cerebral cortex incubated at 38°C but the leakage was more marked in the case of cerebral cortex. The results indicate that, using this criterion of injury, cerebral cortex slices are more sensitive to heat than liver slices. In brain, a marked increase in the concentration of Rb⁶⁺ in the supernatant occurred after incubation for about 1 hr at 42°C, while in liver slices a similar increase required incubation for about 2 hr at 43°C or 1 hr at 45°C.

**CONCLUSIONS**

Several possible methods suitable for the detection of injury in tissues by heat were compared using liver and cerebral cortex slices and the results that appear most informative are plotted in Fig. 1. It is obvious that for a given tissue all these indicators of injury deviate sharply from the control at about the same temperature, 43°C for cerebral cortex and 45°C for liver. The most delicate indicator of damage appears to be the accumulation of ammonia in the medium. This deviates from controls at about 42°C. These findings suggest that similar biochemical changes may play a role in death from hyperthermia.

**REFERENCES**


