Serum Enzymes Following Repetitive Hyperthermia[1,2] (36218)

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Elevation of the body temperature produces bromsulphalein (BSP) retention (1, 2) which is an indication of hepatic injury (3). Since there is a significant extrahepatic uptake of BSP (4), the retarded disappearance of this dye in fever could be partially due to a hyperthermic influence on peripheral tissues.

Increases in the serum enzymes glutamic oxalacetic transaminase (SGOT) and glutamic pyruvic transaminase (SGPT) are considered to be indicative of liver damage as well as myocardial infarction (5), whereas increases in serum isocitric dehydrogenase (ICD) seem to be specific for hepatic injury (6). However, the hemolysis which occurs in hyperthermia (7) makes it difficult to interpret changes in these enzymes during the fever episode because red cells have a high enzyme content (8, 9).

The present experiments on repetitive hyperthermia allowed for blood sampling before and after several bouts of hyperthermia but with the latter sample being taken when the animals were normothermic and free of hemolysis. Therefore, it was possible to study serum enzyme changes resulting from successive inductions of hyperthermia.

Methods. The results to be presented were obtained from experiments on 12 healthy, adult mongrel dogs varying in weight from 16.4–30.0 kg. The animals were fasted approximately 18 hr prior to the experiments and were anesthetized by the intravenous administration of 30 mg/kg pentobarbital sodium. Following anesthetization a No. 8F cardiac catheter was placed in the left hepatic vein under fluoroscopic guidance. The catheter was located in a mid-position to prevent wedging and to minimize sampling of reflux blood from the vena cava. A No. 8F catheter was also placed in the femoral artery for arterial blood sampling. A thermistor probe was inserted approximately 12 cm into the rectum and temperatures measured with a telethermometer.

The dogs were divided into 2 groups of 6 each. The animals in the hyperthermic group were heated to a rectal temperature of 41.5 ± 0.5° by placing them in the rubber blankets of a Thermorite Hypo-Hyperthermia Unit where the circulating fluid had been adjusted to a temperature of 65° (150°F). The animals were wrapped in wool blankets to prevent skin burns. Rectal temperatures increased to 41.5° in approximately 1 hr. Each hyperthermic animal had a normothermic partner, of as nearly the same body wt as possible, which was treated exactly the same as its hyperthermic counterpart except that it was not heated. The 2 dogs were run simultaneously.

Following the first hyperthermic episode the animals were returned to their cages and allowed to cool to normal body temperature. The procedure was repeated on 4 additional days for a total of 5 bouts of hyperthermia. The catheterization and blood sampling procedures were performed on only the first and fifth days.

Arterial and hepatic venous blood for serum enzymes and plasma hemoglobin determinations were taken prior to the first heating and 24 hr after the fourth heating (*i.e.*, after the animals had been anesthetized in preparation for the fifth heating). The normothermic controls were sampled at the same time. ICD

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TABLE I. Plasma Hemoglobin and Serum Enzymes in Six Dogs Before and After Four Bouts of Induced Hyperthermia to a Rectal Temperature of 41.5° and in Six Normothermic Control Dogs.[a]

Day	Normothermia		Hyperthermia	
	Arterial	Hepatic venous	Arterial	Hepatic venous
	Plasma Hemoglobin (mg%)			
First[b]	6.3 ± 2.1	5.7 ± 0.9	4.7 ± 0.5	5.6 ± 0.5
Fifth[c]	4.7 ± 0.8	7.5 ± 2.0	5.9 ± 0.9	7.5 ± 1.0
	Serum Glutamic Oxalacetic Transaminase (Sigma units/ml)			
First	40 ± 9	44 ± 10	24 ± 6	30 ± 10
Fifth	64 ± 16	66 ± 18	102 ± 32[d]	102 ± 34[d]
	Serum Glutamic Pyruvic Transaminase (Sigma units/ml)			
First	25 ± 5	26 ± 5	15 ± 3	22 ± 3
Fifth	57 ± 17	60 ± 16	54 ± 13[d]	55 ± 15
	Isocitric Dehydrogenase (Sigma units/ml)			
First	213 ± 54	234 ± 71	91 ± 17	112 ± 29
Fifth	171 ± 21	201 ± 30	370 ± 87[d]	342 ± 92[d]

[a] Data presented as means ± standard error of the mean.
[b] First experimental day prior to first heating to rectal temperature (T_r) of 41.5°.
[c] Fifth experimental day prior to fifth heating to 41.5° T_r.
[d] $p \lesssim 0.05$ when compared to first experimental day.

was determined by the Sigma[3] modification of the method of Wolfson and Williams-Ashman (9). SGOT and SGPT were measured by the Sigma modification of the method of Reitman and Frankel (10). Plasma hemoglobin was determined by a modification of the benzidine method (11).

Results. The results are all presented as means and standard errors of the mean. The statistical comparisons were made on the basis of paired sample statistics within groups. The null hypothesis was rejected at the 5% level.

The average body wt of the hyperthermic animals did not change significantly during the 5 days of the experiment (21.0 ± 1.9–20.4 ± 2.0 kg), while the normothermic group exhibited a slight decrease (20.5 ± 0.8–19.4 ± 0.8 kg).

The average rectal temperatures at the time of blood sampling on the first and fifth days were 37.4 ± 0.6° and 38.7 ± 0.4°, respectively, in the hyperthermic group and 37.0 ± 0.8° and 37.8 ± 0.6° in the normothermic animals.

The serum enzymes are reported in terms of Sigma units/ml. One Sigma unit of SGOT or SGPT will form 4.82×10^{-4} μM of glutamate/min at pH 7.5 and 25°. One Sigma unit of ICD will form 1×10^{-3} μM/hr of dihydrotriphosphopyridine nucleotide (TPNH) at pH 7.5 and 25°.

The plasma hemoglobin and serum enzyme values in the 2 groups of dogs are presented in Table I. The plasma hemoglobins were at normal levels on the first and fifth days in both groups, indicating that there was no significant hemolysis in the blood samples prior to raising the rectal temperatures of the hyperthermic animals.

The normothermic dogs did not exhibit any statistically significant changes in SGOT, SGPT, or ICD as a result of the repeated anesthetization (Table I). However, in the

[3] Sigma Chemical Company, St. Louis, MO.

hyperthermic animals these enzymes were significantly elevated in arterial and hepatic venous blood on the fifth day, *i.e.*, after four previous heatings to a rectal temperature of 41.5°. The arterial SGOT increased from 24 ± 6 to 102 ± 32 Sigma units/ml ($p < 0.05$) and hepatic venous from 30 ± 10 to 102 ± 34 units/ml ($p < 0.05$). Arterial SGPT increased from 15 ± 3 to 54 ± 13 units/ml ($p < 0.05$) and hepatic venous from 22 ± 3 to 55 ± 15 units/ml. The latter change did not quite achieve statistical significance. Arterial ICD increased from 91 ± 17 to 370 ± 87 units/ml ($p < 0.02$) and hepatic venous ICD from 112 ± 29 to 342 ± 92 units/ml ($p < 0.05$).

Discussion. The normal plasma hemoglobin levels in all blood samples indicate that red cell hemolysis did not contribute significantly to the elevations in the serum enzymes SGOT, SGPT, and ICD seen on the fifth experimental day in the hyperthermic group.

Reed, Manning, and Hopkins (12) measured SGOT, SGPT, and alkaline phosphatase before and following hyperthermic liver perfusion experiments in dogs. They found elevated serum levels of the enzymes on the first postexperimental day. The SGOT rapidly returned towards control values while SGPT and alkaline phosphatase exhibited a more sustained elevation. They did not measure plasma hemoglobin levels and so could not determine if red cell hemolysis could have contributed to their results. However, as in the present experiments, their post-perfusion blood samples were presumably taken from animals with normal body temperatures, although this was not mentioned.

Gupta, Mehrotra, and Agarwal (13) have reported elevated SGPT values in humans 24 and 48 hr after peak temperatures were reached in artificially induced fever. Alkaline phosphatase and other liver function tests were not altered. Increased levels of SGPT were also noted in patients with naturally occurring fevers. However, no indication of hemolysis is mentioned in their paper, although in their patients with artificially induced fever it can be presumed that body temperatures had returned to the normal range.

The results of the present experiments indicate that repetitive hyperthermia results in acute hepatic injury, as evidenced by the elevated SGOT, SGPT, and ICD levels. Since SGOT and SGPT increases are also indicative of myocardial damage (5), while ICD levels appear to reflect only liver damage (6), it is possible that these results could occur from a combination of acute hepatic and myocardial injury. However, myocardial damage from the kind of hyperthermia induced in these experiments has not been reported (14).

Summary. The artificial heating of dogs to rectal temperatures of 41.5° on 4 consecutive days resulted in an increase in the serum enzymes SGOT and SGPT and in isocitric dehydrogenase. Blood samples were taken prior to the first heating and prior to the fifth heating so that body temperatures and plasma hemoglobin values were at normal levels. No significant enzyme changes were noted in a group of control dogs subjected to the same procedures as the hyperthermic group except the repeated heating. The results indicate acute hepatic injury.

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