# Serum Enzyme and Histopathologic Changes in Rats after Cold Exposures. (27256)

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The importance of changes in serum enzyme levels as an aid in diagnosis of various organic lesions is now well recognized(1). It has also been shown that changes in levels of various serum enzymes, such as glutamic oxalacetic transaminase, glutamic pyruvic transaminase, lactic dehydrogenase, alkaline phosphatase and aldolase, occur after severe stress induced by administration of large doses of catecholamines(2), exposure to high altitude(3) and prolonged exercise(4). It has been possible in some instances to correlate these serum enzyme alterations with histologic changes. Recently, hypothermia has been reported(5,6) to produce elevations in serum glutamic oxalacetic transaminase without demonstrable evidence of necrosis or unusual morphologic alterations. This present study was undertaken to determine the effect of exposure of rats to different degrees of cold on a variety of serum enzymes and to correlate such changes with any hematologic and histopathologic alterations.

*Methods.* Groups of adult male Sprague-Dawley rats about 90 days of age housed singly in wire cages, were exposed in a cold room. In Experiments 1 and 2, rats were exposed overnight for 16 hours to 1.7 ± 0.6°C and −5 ± 0.6°C respectively. In Experiment 3, rats were exposed to 1.7 ± 0.6°C for 5 hours on each of 2 successive days; they were immersed in water containing a wetting agent (Alconox) for 1 minute immediately preceding each 5-hour exposure(7). Untreated rats from the corresponding lots were used as controls. The experimental rats exposed to −5°C and the corresponding controls received food but no water during the 16-hour exposure.

Rectal temperatures and tail blood cell counts and hematocrit values of representative animals were determined. Heart blood for serum enzyme studies was obtained during nembutal anesthesia, each rat supplying only one sample, before and at various intervals after the cold exposures (Table I). Values of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), serum lactic dehydrogenase (SLD), serum alkaline phosphatase (SAkP), serum aldolase (SAld) and blood urea nitrogen (BUN) were determined by methods previously described(4). The heart, liver, kidney and thigh muscles of the rats, immediately after they were killed, were fixed in 10% buffered formalin. Paraffin sections were stained with hematoxylin and eosin, and frozen sections were stained for fat with Oil red O. In the first experiment, sections of the muscle and liver of most of the rats were fixed in alcoholic 10% formalin with 5% glacial acetic acid(8) and stained for glycogen by the periodic acid-Schiff procedure; control sections were pretreated with diastase.

*Results. Group 1 (exposed 16 hours to 1.7°C).* Immediately after exposure, there was a significant elevation (Table I) in SGOT ($P<.05$), SAld ($P<.001$) and BUN ($P<.05$). Values returned to normal in 24 hours. Changes in SGPT, SAkP and SLD were not significant. The mean rectal temperature was only slightly reduced (Fig. 1). Mean body weight was reduced 3.9% during exposure and was normal 24 hours later. No rats died. All rats tested showed a decrease in hematocrit and an increase in leukocytes and proportion of neutrophils (Table II).

*Group 2 (exposed 16 hours to −5°C).* Immediately after exposure, the rats had a significant rise in SGOT ($P<.05$), SGPT ($P<.01$), SAld ($P<.05$) and BUN ($P<.001$). BUN was normal the next day and enzyme values were normal in 3 days (Table I). Changes in SLD were not significant. This group had a mean SAkP value of 37 Bodansky units immediately after exposure and a normal value of 115 units the next day. This drop in SAkP is attributed to water deprivation since a similar drop occurred in controls deprived of water. The mean rectal tempera-

TABLE I. Mean Serum Enzyme and Blood Urea Nitrogen Values in Rats Exposed to Cold.

| No. of rats | Days after end of treatment | SGOT, U/ml | SGPT, U/ml | Serum aldolase, U/ml | BUN, mg/100 ml |
|---|---|---|---|---|---|
| | | Exp. 1. Rats exposed 16 hr to 1.7°C | | | |
| 9 | Controls | 274 ± 115 | 72 ± 22 | 42 ± 28 | 30 ± 5 |
| 13 | 0 | 367 ± 88 | 78 ± 12 | 105 ± 59 | 41 ± 13 |
| 6 | 1 | 303 ± 38 | 75 ± 6 | 52 ± 11 | 29 ± 2 |
| 4 | 3 | 211 ± 77 | 60 ± 9 | 44 ± 8 | 26 ± 1 |
| | | Exp. 2. Rats exposed 16 hr to -5°C | | | |
| 6 | Controls | 380 ± 51 | 79 ± 17 | 44 ± 16 | 24 ± 5 |
| 6 | 0 | 436 ± 33 | 103 ± 11 | 138 ± 73 | 42 ± 7 |
| 6 | 1 | 437 ± 20 | 99 ± 8 | 134 ± 40 | 26 ± 4 |
| 4 | 2 | 426 ± 38 | 90 ± 10 | 119 ± 65 | 31 ± 4 |
| 2 | 3 | 336 ± 59 | 85 ± 8 | 66 ± 14 | 28 ± 3 |
| | | Exp. 3. Rats wet before 5-hr exposure to 1.7°C on 2 successive days | | | |
| 9 | Controls | 332 ± 48 | 63 ± 10 | 76 ± 11 | 25 ± 4 |
| 6 | 0 | 452 ± 14 | 74 ± 17 | 167 ± 62 | 37 ± 5 |
| 7 | 1 | 550 ± 75 | 67 ± 9 | 193 ± 57 | 22 ± 3 |
| 6 | 2 | 363 ± 32 | 64 ± 7 | 145 ± 63 | 28 ± 5 |
| 6 | 3 | 408 ± 39 | 73 ± 11 | 180 ± 115 | 24 ± 1 |
| 6 | 6 | 350 ± 83 | 63 ± 7 | 89 ± 31 | 26 ± 3 |

Mean values ± stand. dev. of serum glutamic oxalacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum aldolase are given in units/ml of serum. Values of blood urea nitrogen (BUN) are given in mg/100 ml of blood. Blood obtained by cardiac puncture.

ture fell moderately (Fig. 1). Mean loss in body weight during the exposure was 6.5%. Most rats recovered their normal weight within one day, several required 2 days, while one lost weight for 5 days. Hematologic changes were similar to those of Group 1 (Table II). The shift in differential count in both groups persisted 6-24 hours.

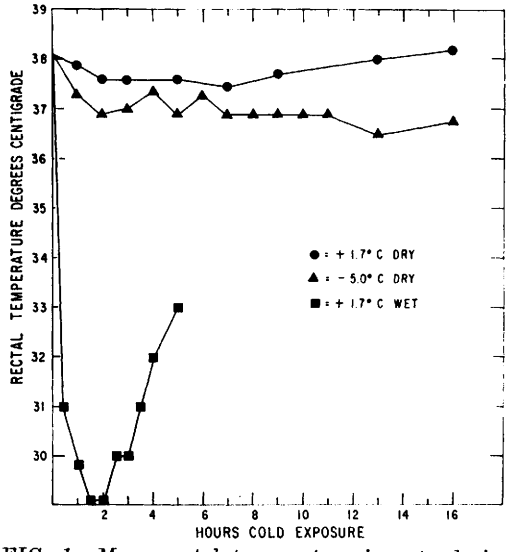


FIG. 1. Mean rectal temperature in rats during cold exposures.

TABLE II. Hematocrits and Total and Differential Leukocyte Counts in Rats before and after Cold Exposures.

| | Group 1 | Group 2 | Group 3 |
|---|---|---|---|
| | Hematocrit values | | |
| Before exposure | 45 ± 4 | 48 ± 2 | 49 ± 4 |
| After " | 40 ± 4 | 43 ± 4 | 49 ± 5 |
| | Total leukocytes/mm³ × 10³ | | |
| Before " | 15 ± 6 | 22 ± 5 | 21 ± 8 |
| After " | 50 ±11 | 40 ± 8 | 19 ± 7 |
| | Lymphocytes (%) | | |
| Before " | 82 ± 5 | 84 ± 3 | 85 ± 8 |
| After " | 66 ±11 | 54 ±15 | 78 ±11 |
| | Neutrophils (%) | | |
| Before " | 13 ± 5 | 11 ± 3 | 8 ± 7 |
| After " | 29 ±10 | 41 ±15 | 16 ± 9 |

Groups 1 and 2 each included 7 rats exposed 16 hr to 1.7 and -5°C respectively and Group 3 included 5 rats wet before 5-hr exposure to 1.7°C on 2 successive days. Mean values ± stand. dev. are given on tail blood obtained on same rats before and 0-2 hr after exposure.

*Group 3 (wet before exposed 5 hours to 1.7°C on 2 successive days).* Immediately after the 2nd exposure, there was a significant rise in SGOT ($P<.01$). SAld ($P<.01$) and BUN ($P<.001$). SGOT and SAld values reached their peak the next day and were normal in 6 days (Table I). BUN values were normal in 24 hours. Changes in SGPT,

SAkP and SLD were not significant. The mean rectal temperature during each exposure fell to about 29°C within 2 hours (Fig. 1). In 25% of the rats, rectal temperature continued to decline during one of the 2 exposures until death in 2-3 hours. Mean loss in body weight after each exposure was about 3% and recovery was slow. Mean body weight of 6 rats was 3% below preexposure levels at the end of 6 days. In contrast to Groups 1 and 2, the hematologic changes in Group 3 varied in different rats and were not significant (Table II).

*Pathologic findings.* Paraffin sections revealed no significant changes clearly attributable to the cold exposures. In the first experiment, glycogen stains disclosed nearly complete depletion of glycogen in rat liver and partial depletion in the muscle immediately after the 16-hour exposure to 1.7°C. Glycogen content appeared normal in the liver of rats killed 24 hours and in the skeletal muscle of rats killed 48 hours after the end of the cold exposure.

In all 3 experiments, frozen sections stained for fat revealed slight to moderate depletion of lipid in the adrenal cortex in most of the rats killed immediately after the cold exposures. In addition many rats showed fatty changes in liver, kidney, or heart. The fat was stained with Oil red O and was dispersed as fine droplets in the liver cells, chiefly in the periportal zones, at the base of the epithelial cells lining the renal convoluted tubules, and in myocardial fibers in patchy areas. The depletion of lipid in the adrenal cortex and the fatty changes in the viscera were rarely seen in rats killed more than 24-48 hours after the exposures.

Fatty changes in the liver were moderate to severe in the rats 90 days old killed immediately after exposure to −5°C; only 2 of 6 rats 59 days old showed such changes. In rats killed immediately after exposure to 1.7°C, slight to moderate fatty changes in the liver were seen in about half of the rats that were wet before exposure (Exp. 3) and in only one of the dry rats exposed to 1.7°C (Exp. 1).

Fatty changes in the kidney, slight to moderate in degree, were seen only in rats 90 days old killed immediately after exposure to −5°C. Fatty changes in the myocardium were slight and were seen in about half of the wet rats killed immediately after the 2nd exposure to 1.7°C (Exp. 3) and less frequently in the other 2 experiments. In all 3 experiments, there were occasional striated muscle fibers that contained some fine fat droplets; however, there was no significant difference between experimental and untreated control rats in the incidence or severity of these slight fatty changes in the muscle. There was no close correlation between the severity of fatty changes in the organs studied and degree of elevation of the serum enzymes.

*Discussion.* Exposure of rats to various degrees of cold produced a significant transient rise in SGOT, SAld, and BUN. Previously, Wendel(5) and Blair, Hood, Tolley and Bunce(6) had demonstrated an increase in SGOT after hypothermia in dogs. It is now evident that SGOT and SAld increase in rats immediately after exposure to cold even in the absence of severe hypothermia. However, increases in SGOT and SAld were greater and more persistent in the wet rats exposed to 1.7°C, a procedure which produced a more profound hypothermia. This suggests that the more severe the hypothermia the greater the elevation in serum enzymes.

Wendel(5) and Blair, Hood, Tolley and Bunce(6) noted no histologic changes in hypothermic dogs which had shown a rise in SGOT(5,6), but reported no studies for glycogen or fat in their dogs. However, Knocker (9) earlier reported fatty changes in the liver and kidney of hypothermic dogs and glycogen depletion in the liver. In this study, no histologic changes clearly attributable to the cold exposures in the 3 experiments were found in routine paraffin sections stained with hematoxylin and eosin. However, stains for glycogen revealed transient depletion of glycogen in liver and muscle, and stains for fat revealed transient fatty changes in liver, kidney and heart and depletion of lipid in the adrenal cortex. Similar but more severe fatty changes, glycogen depletion, and serum enzyme elevations were reported previously in rats following prolonged exercise(4). This

suggests that some of our findings may be attributable in part to muscular activity associated with shivering due to the cold exposures.

The fatty changes in the organs indicated cellular damage. However, the occurrence of serum enzyme elevations in animals recovering from or showing no significant fatty changes indicated that a less severe cellular alteration, not readily demonstrable histologically, may have been responsible for the elevations. Changes in permeability of mitochondria and cellular membranes, permitting enzymes to escape and accumulate in the blood, have been considered as factors increasing serum enzyme levels after certain stresses(2,3,4,6). It is postulated that cellular permeability increases in rats after cold exposure even in the absence of marked hypothermia and accounts in part for the rise in serum enzymes observed. The concomitant depletion of adrenal lipid and other fatty changes and changes in total and differential leukocyte counts suggest that the adrenal hormones may also play a role. However, many other changes, such as altered thyroid function and rise in serum magnesium, have been reported in hypothermic animals(10,11) and may be contributing factors to the findings reported here.

The rise in BUN is attributed in part to transient renal damage evidenced by the fatty changes. Steadman, Ariel and Warren(10) noted anuria in hypothermic rabbits but did not report BUN values.

*Summary.* Exposure of 2 groups of dry rats 16 hours to 1.7 and −5°C and a third group of wet rats 5 hours to 1.7°C on 2 successive days significantly increased levels of serum glutamic oxalacetic transaminase, serum aldolase and blood urea nitrogen. The blood urea nitrogen in all 3 groups and serum enzyme values in the 1st group were normal in 24 hours. The serum enzyme values in the 2nd and 3rd groups were normal in 3 and 6 days respectively. Mean body temperatures in the 3 groups at the end of the cold exposures were 38.2, 36.7 and 33°C respectively. The transient rise in serum enzymes coincided with a transient decrease in mean hematocrit and in glycogen in liver and muscle and lipid in the adrenal cortex, and leukocytosis. Many rats also showed transient fatty changes in the liver and, less frequently, in the kidney and heart.

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