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Serum Hexosaminidase Activity in Man Under Simulated Diving Conditions[1] (38523)

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Several enzyme activities have been studied in the sera of animals and humans exposed to a variety of hyperbaric conditions (1–4). The metabolic defect in a number of sphingolipid storage diseases known today is an attenuation or complete absence of a specific hydrolytic enzyme required for the catabolism of a lipid (5). Our previous results showed that there were changes in the amounts of sphingoglycolipid in the blood and tissues of rats following hyperbaric exposure (6). Therefore, an investigation of enzyme activity associated with sphingoglycolipid metabolism in the serum of human divers was initiated. Hexosaminidases were measured, since there is a considerable amount of hexosaminidase A and B activity in serum which can be quantitatively measured with artificial substrates (7).

Materials and Methods. All dives were conducted in the hyperbaric chamber complex of the Naval Experimental Diving Unit, Washington, D.C. The following dives were performed:

Saturation dive at 1000 feet of sea water (FSW). Four subjects were compressed at a rate of 5 ft/min on a mixture of helium and oxygen to an ultimate saturation depth of 1000 FSW. During compression, three day intermediate stops were made at 200, 400, 600 and 800 FSW. The divers remained four days at a saturation depth of 1000 FSW. Decompression was performed in accordance with the standard U.S. Navy format (Table I), with the exception of a 24 hr stop at 850 FSW to permit physiological studies.

Subsaturation dives at 650 FSW. Fourteen divers, in three successive dives, were compressed on a helium and oxygen mixture to a simulated depth of 650 FSW at a rate of 30 ft/min to 400 FSW, 15 ft/min from 400 to 600 FSW, and 10 ft/min from 600 to 650 FSW. Following a 3.5 hr holding period at this depth, rapid decompression was performed. In all three dives, however, the initial rapid decompression resulted in decompression sickness at a depth of 430–470 FSW in one or more subjects, necessitating therapeutic recompression to 650 FSW and overnight saturation at that depth. Subsequent decompression from 650 FSW was performed in accordance with the schedule in Table I.

Subsaturation dive at 400 FSW. Eight divers were exposed to a mixture of helium–oxygen at a simulated depth of 400 FSW on two successive dives. After a 3.5 hr holding period the chamber was decompressed on an experimental schedule.

During these helium–oxygen dives, the chamber atmosphere was monitored continuously for oxygen and carbon dioxide content, temperature and relative humidity. Oxygen concentration was maintained between 0.29 and 0.35 atmosphere, carbon dioxide content was not allowed to exceed 0.5 % surface equivalent, temperature ranged from 26° to 32°, and relative humidity from 50 to 70 %.

Fasting blood samples were obtained by venipuncture at 7 AM on the days indicated (Figs. 1–3). The blood samples that were drawn at increased ambient pressure were decompressed at a rate of 15 ft/min. Prior to centrifugation, samples were stored in an ice bath. After clotting, samples were centrifuged at 500 *g* for 5 min at room temperature and the serum was separated.

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TABLE I. RATE OF DECOMPRESSION FROM SATURATION EXPOSURES ON HELIUM–OXYGEN.

Depth (ft sea water)	Rate[a] (ft/hr)
Initial 30 ft ascent	10
1000–200	6
200–100	5
100–50	4
50–Surface	3

[a] Decompression is interrupted daily between 1400 and 1600 hr and between 0000 and 0600 hr.

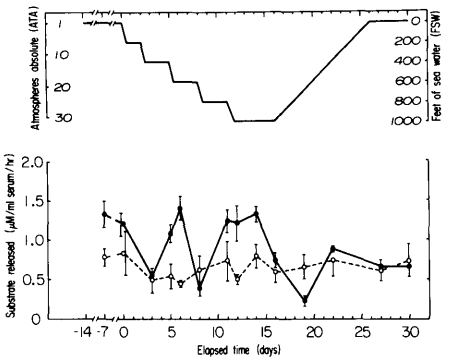

FIG. 1. Serum hexosaminidase levels during a 1000 FSW saturation dive. Upper section: diving schedule; lower section: enzyme activity, hexosaminidase A (●); hexosaminidase B (○), values are the mean ± standard error.

Hexosaminidase A and B activities were measured by the procedure of O'Brien (7). Total hexosaminidase activity was measured using 10 μl of serum diluted with 50 μl of 0.1 *M* citrate–phosphate buffer (pH 4.4). One hundred μl of 10 m*M* 4-methylumbelliferyl-*N*-acetyl-β-glucosamine (Sigma Chemical Co., St. Louis, MO) dissolved in the buffer described above was added to each sample. After incubation at 37° for 30 min, 5 ml of 0.17 *M* glycine–NaOH buffer (pH 10) was added to stop the reaction. Fluorescence was determined in an Aminco–Bowman spectrophotofluorometer at an excitation wavelength of 366 nm and an emission wavelength of 446 nm. To measure hexosaminidase B activity, the same conditions were used as described above except the serum was preincubated for 4 hr at 50° to inactivate hexosaminidase A selectively. A standard curve was determined using 4-methyl-umbelliferone dissolved in 0.17 *M* glycine–NaOH buffer over a range of 0–10 nmoles. The extent of substrate cleavage was determined by comparison of the fluorescence of the samples against the standards. All samples were measured in duplicate. The activity of hexosaminidase A was calculated as the difference in total activity and that activity remaining after heating at 50° for 4 hr.

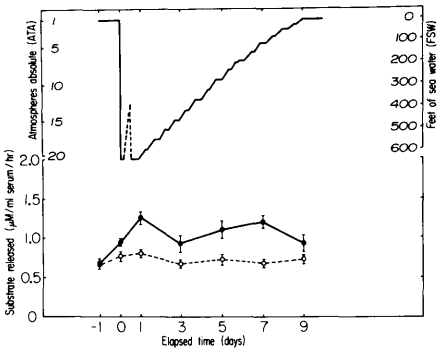

FIG. 2. Serum hexosaminidase levels during 650 FSW subsaturation dives. Upper section: diving schedule; lower section: enzyme activity, hexosaminidase A (●); hexosaminidase B (○), values are the mean ± standard error.

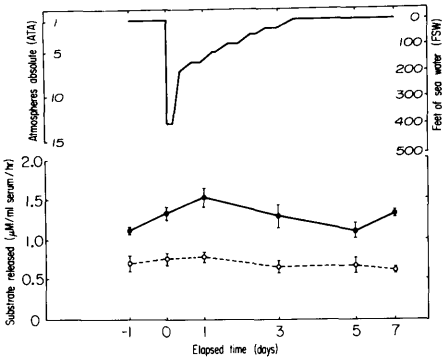

FIG. 3. Serum hexosaminidase levels during a 400 FSW subsaturation dive. Upper section: diving schedule; lower section: enzyme activity, hexosaminidase A (●); hexosaminidase B (○), values are the mean ± standard error.

Results. One thousand FSW dive. The activities of hexosaminidases A and B in the sera of divers in a 1000 FSW saturation dive are shown in Fig. 1. All four divers had very similar patterns of change. The mean activity of hexosaminidase A decreased more than 50% at the end of compression from 200 FSW to 400 FSW and returned gradually to normal, then dropped again at 600 FSW.

The enzyme activity remained in the normal range at 800 FSW and in the first part of the 4-day saturation period at 1000 FSW. A further decrease in hexosaminidase A activities was observed at the end of the 4-day saturation period at 1000 FSW and in the early stage of decompression. There were no remarkable changes in the mean values of hexosaminidase B.

Six hundred-fifty FSW dive. Figure 2 shows the changes in the mean value of serum hexosaminidase activities during the 4-hr subsaturation dives at 650 FSW. Ten of the divers reported compression arthralgia and all divers reported symptoms of the high pressure nervous syndrome, including tremors and unsteadiness. During the initial decompression at 650 FSW, four of the fourteen divers developed severe decompression sickness requiring therapeutic recompression at 650 FSW and overnight saturation. There were increases in mean values of hexosaminidase A activities at the depth of 650 FSW after recompression and at the depth of 300 FSW and 100 FSW during decompression, whereas no changes were found in the mean values of hexosaminidase B activities.

Four hundred FSW dive. Figure 3 illustrates the hexosaminidase activity for the 400 FSW dive. A slight increase in hexosaminidase A activities was observed at a depth of 150 FSW during decompression. Hexosaminidase B activities fluctuated within the normal range.

Discussion. Earlier studies showed that hexosaminidases are enzymes responsible for catabolism of ganglioside GM_2 and globosides and can be used as a marker in sphingolipid storage diseases (5). Changes in glycolipid metabolizing enzymes associated with hyperbaric exposure have not been investigated to our knowledge. In this study, an increase in hexosaminidase A activity was observed 24 hr after the compression–decompression cycle in the 400 FSW and 650 FSW dives. In contrast, in the 1000 FSW dive a marked decrease in hexosaminidase A activity was observed at 400, 600 and 1000 FSW during compression and during the early stages of decompression. The functional changes that may occur in cells or tissues related to the observed changes in serum enzyme activities at extreme depths cannot be explained at this time. We cannot account for the widely differing profiles of our observations in the three dives. It appeared that the changes might be related to the single or combined effects of increased ambient pressure, compression or to the effects of decompression. Also, individual responses of the subjects can vary greatly. Uddin *et al.* (3) have reported that changes in serum creatine phosphokinase (CPK) activity were observed during hyperbaric exposure and suggested that both compression and decompression stress, if sufficiently severe, may produce elevations in CPK activity.

The procedures for measuring glycolipid metabolizing enzymes are sensitive enough to permit detection of minor changes of serum enzyme activity under high stress conditions. However, the site of action, amplification of the observed responses and the functional changes related to the enzyme activity, and lipid changes remains to be determined.

Summary. The activities of serum hexosaminidases from human divers before, during and after simulated dives was measured. Decreases in hexosaminidase A activities were observed in the 1000 FSW saturation dive, whereas an increase in hexosaminidase A activity was observed during decompression in the subsaturation dives at 400 FSW and 650 FSW.

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The opinions and statements contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

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