Effects of Hypobaric Hypoxia on Serotonin in Mouse Brain

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EFFECTS OF HYPOBARIC
HYPOXIA ON SEROTONIN
IN MOUSE BRAIN

by

Jacob Dale Peuler

A Thesis
Submitted to the
Faculty of The Graduate College
in partial fulfillment
of the
Degree of Master of Arts

Western Michigan University
Kalamazoo, Michigan
August 1975
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Special appreciation is expressed to Dr. Garland A. Johnson, whose instruction, guidance and encouragement will long be remembered.

Dr. Clarence J. Goodnight and Dr. Jean M. Lawrence contributed many hours of instruction and personal concern in the preparation of this thesis, for which heartfelt thanks are offered.

Jacob Dale Peuler
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INTRODUCTION

The effects of altered environmental conditions on serotonin (5-hydroxytryptamine) in the central nervous system has been a subject of continued study since its discovery in brain tissue by Twarog and Page (1953). The influence of such factors as ambient light and temperature on brain serotonin has been extensively investigated in numerous animal species (Page, 1968; Simmonds, 1970). Very little study, however, has been directed toward the influence of another equally important environmental factor—ambient barometric pressure.

Many organisms, particularly terrestrial animals living near sea level, are physiologically sensitive to hypobaric stress—i.e., significant reduction in barometric pressure below one atmosphere (760 mmHg). The responses to this stressful condition are in most instances considered adaptations to reduction in only the partial pressure of atmospheric oxygen (Hoar, 1966). As such, the condition is often referred to as hypobaric hypoxia or anoxia. The effect of hypobaric (high-altitude) hypoxia on brain serotonin has only been investigated in the rat. Early studies involving exposure to extremely low oxygen levels for only a few minutes reported no significant alteration of serotonin levels (Garattini, Kato, and Valzelli, 1958; Anderson and Bonnycastle, 1960). However, a recent study in which rats were exposed to a high-altitude for a longer period of time (days) reported a significant elevation of serotonin in the cerebral hemispheres and a slight but significant reduction in hypothalamic serotonin (Rutsai and Meerson, 1973). In another
recent study turnover of brain serotonin was reported to be significantly reduced in rats during severe hypoxia (Davis and Carlsson, 1974).

A reduced oxygen level in the gaseous environment is eventually communicated to the cellular level of the organism via reduced oxygen tension in the bloodstream. Anoxia at the level of the cell may also be produced by occlusion of blood vessels. Such ischemic anoxia has been reported to influence cerebral blood levels of serotonin in baboons during and after occlusion of selected arteries (Welch, Meyer, Feranra, Hashi and Shinmaru, 1972). The authors suggested that the observed changes may be due to movement of serotonin across the blood-brain barrier which may have been damaged by the anoxic condition.

In view of these findings, the present study was undertaken to determine the effects of chronic hypobaric hypoxia on (1) content and turnover of serotonin in mouse brain, and (2) brain levels of radioactive serotonin administered intravenously to mice, post-exposure.
LITERATURE SURVEY

Hypobaric Chambers

Numerous environmental chambers have been developed for investigating the physiological effects of hypobaric hypoxia in small animals. The majority of systems involved decompression of a heavy-walled chamber with a vacuum pump. In addition, a metered intake of air was required for prolonged exposures. A critical problem encountered in the design of these systems was the control of vacuum and intake rates such that a constant internal pressure is maintained. Rolls and Loevenhart (1915) constructed a chamber with electromagnetic regulation of a special air-intake valve. Later Loehning, et al., (1953) modified this valve so that it operated on the principle of the automatic tire valve. Many animal experiments were conducted with this system (Loehning, et al., 1953; Van Liere, 1942; Valdivia and McFee, 1957). However, it was required that the construction of the special intake valve be done in the laboratory since it was not commercially available. Kollias and Jordan (1965) eliminated the need for the tire-valve design by introducing the bypass-arm design which provided for auxiliary vacuum and intake rates whenever needed. The bypass arms—one on the vacuum line and the other on the intake line—were controlled by solenoid valves which in turn were regulated by a modified altimeter. The altimeter was reconstructed to include two electrical switches which were activated by either high or low deviation for a selected altitude. This system at least allowed for the use of commercial flow-
Serotonin

The effects of hypobaric hypoxia on brain serotonin levels has only been examined in rats. In one study involving 10 minutes of exposure to pure oxygen at 10mmHg (total pressure) an increase in serotonin was observed in every region of the brain examined. Those regions examined were the cerebrum, cerebellum, diencephalic zone, mesencephalic zone, and whole brain (Garattini, Kato, Valzelli, 1958). However, none of the elevations—ranging from +3% to +57% of control—were statistically significant. The authors suggested this was due to increased variability of the brain serotonin in the exposed rats.

In another study involving a more tolerable level of hypoxia—10% oxygen in one atmosphere total pressure—no influence on serotonin content was reported. Here the duration of the exposure was 20 minutes (Anderson and Bonnycastle, 1960). In a more recent study, rats were exposed to a simulated altitude of 19,500 ft. for 40 days (6 hours daily). The concentration of oxygen at that altitude is equivalent to that of 10% oxygen in one atmosphere total pressure. The investigators found a significant 18% elevation of serotonin in the cerebral hemispheres and a significant 8% reduction in hypothalamic serotonin after the exposure (Rutsai and Meerson, 1973). They suggested that the cerebral elevation may play a significant role in the mechanism whereby adaptation to hypoxia prevented audiogenic epileptiform convulsions.
Davis and Carlsson (1974) examined brain serotonin turnover in rats during exposure to an oxygen-nitrogen mixture of 5.6% oxygen at a total pressure of one atmosphere. Turnover was measured by two different methods. One method involved the determination of the rate of accumulation of serotonin with time after administration of a monoamine oxidase inhibitor (pargyline) which blocks the intraneuronal degradation of serotonin. The second method involved the determination of the rate of accumulation of 5-HIAA (the major metabolite of serotonin within the cell) with time after administration of probenicid which blocks the active transport of this metabolite from the cell (Neff and Tozer, 1968). The blocking agents were administered just prior to exposure to the low oxygen mixture. After an unspecified duration of exposure (possibly one hour) the rats were sacrificed and their whole brains analyzed for the respective accumulations. Turnover as represented by the rates of serotonin accumulation after pargyline was less than 40% of control while that represented by the rate of accumulation of 5-HIAA after probenicid was less than 10% of control. The authors suggested that these data demonstrated the dramatic influence of severe hypoxia on both synthesis and oxidative degradation (deamination) of serotonin within the neuron.

The effects of environmental hypoxia on serotonin in two other organs—lungs and intestine—have been examined in guinea pigs by Garcia-Perez and Lucas-Gallego (1967). Lung serotonin was significantly elevated while intestinal levels were reduced. Also serotonin excretion in these animals was reduced.
Blood levels of serotonin during and after acute hypoxia have been examined in dogs and baboons. Nayar, Mathur, and Ranade (1972) observed no differences in blood levels associated with the pulmonary circulation in dogs which were breathing an oxygen-nitrogen mixture of 10% oxygen at one atmosphere of pressure for less than one hour. Welch et al. (1972) demonstrated significant differences in cerebral venous blood levels of serotonin during and after ischemic anoxia in baboons. In comparison to cerebral arterial blood, venous blood levels were elevated during the ischemic condition and decreased afterwards. The latter condition persisted for at least one hour post-ischemia. The authors suggested that serotonin was being released from the brain into cerebral venous blood during ischemia and was being taken up from the blood into the brain after the ischemia. They postulated this may be due to increased permeability of the blood-brain barrier which may have been damaged by the anoxic condition.
MATERIALS AND METHODS

Altitude Simulation Unit

Principles of Operation. A continuous-duty environmental chamber capable of simulating high-altitude conditions was designed and constructed for the hypobaric exposures in this study. Prolonged, high altitude simulation (chronic hypobaricity) was properly produced by performing three operations, successively: 1) partial evacuation of air from a vacuum-tight chamber to obtain the desired altitude/pressure, 2) admittance of room air into the system to neutralize the effect of animal respiration on relative O₂/CO₂ levels, and 3) balance of evacuation rate against inflow rate to hold the desired altitude/pressure in steady-state equilibrium. In addition, safety devices were automatically activated to prevent large, potentially dangerous deviations from the equilibrium position. Other factors such as light and temperature were subject to appropriate maintenance operations in both test and control facilities.

Vacuum Source. Partial evacuation and continued removal of air from the chamber were accomplished by a continuous-duty, two-stage vacuum pump (Hyvac 14, Cenco Instruments Corp., Chicago, Ill.) capable of displacing 140 liters of free air per minute. The pump was equipped with a ballast valve which, if open during prolonged operation, minimizes the level of air contamination due to uptake of water vapor. In addition, an air drying unit (model 4001-2, Wilkerson Corp., Englewood, Colorado) filled with cotton and desiccant (silica
gel) was placed in the vacuum line immediately prior to the pump to remove dust and a large fraction of water vapor.

Exhaust from the pump was piped from the room by way of a large-diameter exhaust line. Oil mist discharged from the pump's exhaust port collected in a baffle directly above the outlet and ran back into the pump (Plate I, page 37).

**Test Chamber.** Structural details of the chamber are shown in Plate VII (page 43). The unit consisted of a double-walled stainless steel cylinder formed and welded by Kalamazoo Sheet Metal Mfg. Co., Kalamazoo, Mich., a clear plastic window, and a door-lock assembly removed from the shell of a discarded autoclave. The window and door-lock were fitted onto extensions of the heavy, inner wall of the cylinder.

The window—a thick plexiglas plate—was bolted to a flat stainless steel flange welded to the rim of one end of the inner wall. A flat gasket cut from a thick sheet of neoprene was inserted peripherally between the plastic window and the metal flange to create a vacuum seal. The center of the window was cushioned against a steel cross beam to prevent collapse at very low interior pressures.

The door-lock assembly, originally part of an upright autoclave (Precision Scientific 67012, S/P no. A9110), consisted of the following components: a heavy, 14"-dia. brass ring, a cast-aluminum cover hinged to the ring, a resilient "O" ring gasket fitted between the ring. The brass ring was riveted to the other end of the cylinder's inner wall and the brass-to-stainless steel joint was leaded to ensure a vacuum seal.
Three stainless steel pipe couplings (1/4" NPT) were welded into holes drilled in the inner cylindrical wall near the window to accommodate an air intake circuit and two animal watering lines. Existing outlets in the door cover that originally supported apparatus for autoclaving were rethreaded to accommodate electrical connects for interior lighting, and pneumatic circuits to the pump and other units. Two threaded ports in the outer cylindrical wall of the chamber allowed circulation of water through the enclosed jacket.

Control and readout units were mounted on an instrument panel (Plate IV, page 40) above the chamber which in turn was cradled in a movable wooden frame (Plate II, page 38 and Plate III, page 39).

The metal shell of the salvaged autoclave from which the door-lock assembly was removed served as a room-altitude/room-pressure control chamber.

**Pneumatic System.** The complete pneumatic system is shown in Plate VIII (page 44). Two types of pneumatic circuits—static-pressure and air-flow—are schematically illustrated. All circuits were constructed with stainless steel pipe fittings (3/8" ID, 1/4" NPT) and tygon vacuum tubing (1/4" and 3/8" ID's, formula R-3603). Fitted hose clamps were used to ensure vacuum-tight connections. Stainless steel pipe unions (1/4" NPT) served as excellent quick-disconnectors for portable units.

Direct read-out of simulated altitude in the test chamber was obtained from an aircraft altimeter as suggested by Kollias and Jordan (1965). The altimeter (sensitive Kollsman 71-343-8, Aircraft Components, Inc., Benton Harbor, Mich.) communicated with the test
chamber through a static-pressure circuit. Given the altitude read-out, total chamber pressure was easily obtained from a standardized calibration curve (pressure vs. altitude).

Another total pressure estimate could be calculated using a differential pressure read-out obtained from a unique, high-low limit, control instrument—Photohelic pressure-switch/gauge (model 3215, F.W. Dwyer Mfg. Co., Michigan City, Indiana). The gauge component of this instrument consisted of two pressure-tight compartments separated by a flexible diaphragm which exhibited linear motion in response to pressure differentials. The pressure-indicating pointer on the gauge read-out scale (0-15 psi) was connected to the diaphragm via special "magnetic" linkage. This allowed a chatter-free read-out of differences in pressure between the two compartments. The "low" pressure compartment communicated with the test chamber through the same static-pressure circuit used for the altimeter. The "high" pressure compartment was connected by another static line to a stoppered, constant-temperature, vacuum flask partially filled with desiccant. This flask (1000 ml) functioned as a reference pressure which could be opened or closed to the room via glass stopcock (Plate II).

Two separate air-flow circuits—intake and vacuum—joined control chamber to test chamber and test chamber to vacuum pump, successively. Under normal operating conditions, room air entered the control chamber, equally ventilated both chambers, and finally returned to the atmosphere via pump exhaust. The rate of ventilation was monitored by flowmeters (Rate Master RMA-6, F.W. Dwyer Mfg. Co.)
located in the room-pressure portion of the intake circuit.

Another section of intake line directly adjacent to the test chamber was split into two parallel arms (Kollias and Jordan, 1965). One was equipped with a stainless steel needle valve to allow regulation of the normal air intake; the other with a solenoid (electrical) shutoff valve in series with a needle valve to provide an additional bypass flow if needed. A similar alteration of the vacuum circuit provided control of normal and auxiliary vacuum rates. When the intake circuit was closed, the test chamber could be evacuated to a pre-determined altitude/pressure by simply opening the vacuum rate valve. Ventilation was initiated by opening the intake rate valve. To hold the desired altitude/pressure in equilibrium and to maintain the initial ventilation rate required simultaneous readjustment of both intake and vacuum rates. In order to simplify this operation, the original bypass-arm design developed by Kollias and Jordan (1965) was modified in terms of valve positions. The improved arrangement is shown in Plate VIII.

The stainless steel needle valves (3/16" dia. orifice, $C_v = 0.41$) used in this system were selected for precise and reliable flow regulation. Each valve was equipped with a long, tapered stem. The special contour of the stem permitted virtually linear flow control over 80% of the metering range. The valves (model M92T1-2PP, precision hard seat needle) were obtained from Circle Seal Products Co., Anaheim, California.

The solenoid valves (model ESM-2301-120-3, 5 amp, direct actuation) were obtained from Versa Products Co., Englewood, New
Jersey. These valves provided full flow when opened and a leakproof seal when closed. The tip of the solenoid stem was fitted with a hard, Kel F washer which sat firmly against a stainless steel orifice (3/16"-dia., \( C_v = 0.56 \)).

**Altitude/Pressure Safety Control.** During prolonged operation various factors—pump-oil contamination, room barometric pressure, reduced line conductances, etc.—did affect change of the altitude/pressure level. Significant deviation from the initial equilibrium position could, however, be arrested by activating (opening) the appropriate bypass solenoid valve. When the solenoid was open, the speed with which the deviation was counteracted was regulated by the bypass rate valve in series with the solenoid.

The solenoid valves were wired to selected relay terminals at the rear of the Photohelic pressure-switch/gauge (Plate IX, page 45). The standard Photohelic instrument was a combination of the gauge component described in the previous section and two independent, pressure-sensitive "switches". Each "switch" consisted of a DPDT slave relay (10 amp, 110 volt) operated by an amplified photocell circuit. The photoelectric cell was mounted at the base of a set pointer which could be manually adjusted to any position on the gauge read-out scale. The photocell circuit was broken whenever the base of the pressure-indicating pointer interrupted the light to the cell. The resulting signal triggered the slave relay which in turn activated the solenoid. Hence, movement of the pressure-indicating pointer to either the right or left would open one or the other bypass solenoid valves—provided the two, photocell-mounted, set
pointers were properly positioned about the pressure pointer. This operation was used to establish "high" and "low" safety limits for the altitude/pressure level in the test chamber. The resulting safety "interval" could be shifted anywhere about the desired level.

Successful safety control required that the bypass solenoid valves be wired correctly to the appropriate relay terminals of the Photohelic unit. The proper connections are schematically illustrated in Plate IX. Manual activation switches included in the diagram were mounted on the instrument panel (Plate IV).

**Temperature Control.** Water from a reservoir (Plate III) was circulated through the jacket of the test chamber to regulate interior temperature. The temperature was monitored with a mercury thermometer positioned inside and near the window. The outer wall of the jacket was wrapped with asbestos and heat-duct tape to minimize heat exchange with the room. A Bronwill CTC (model 30131, Will Scientific, Inc.) was used to control the temperature and pump the water (12 liters per minute). A check valve placed in the inflow line prevented the jacket from emptying in the event of pump failure (Blatteis and Tucker, 1961).

**Light Control.** Artificial light within the test chamber (Plate V, page 41 and Plate VI) was set on a daily cycle with a 24-hour time-switch (Plate III). Light from the room could be blocked from the chamber interior by simply fitting the outside of the window with a sheet of aluminum foil.

**Watering System.** Animals within the test chamber obtained water through a special, one-way, drinking valve (LV-100, Unifab Corp.,
Kalamazoo, Michigan). The valve was fixed at the base of a water reservoir inside the chamber. The top of the reservoir was open to the chamber atmosphere to allow pressure equilibration. The reservoir was filled through a water line which was equipped with a glass stopcock outside the chamber (Plate VI).

Serotonin Studies

**Exposure Procedures.** Male CF#1 mice (20-22gm) were used in all hypobaric exposures in this study. All tests were conducted between 22,000 and 23,000 ft. simulated altitude (approximately 300 mmHg total pressure). During each exposure, a second group of mice was housed in the room-pressure control chamber. Both test and control groups were maintained at the same environmental temperature (25°C) and light cycle (12-hour light/12-hour dark) and allowed food and water ad libitum.

In three separate experiments involving 24, 48, and 72 hours of exposure to hypobaric hypoxia, whole brain levels of serotonin were determined immediately (within 15 minutes) after terminating the exposures. In another experiment involving 28 hours of exposure, whole brain levels of serotonin were determined at 0.25, 1, 2, 4, 8, 16, and 30 hours post-exposure. Serotonin levels in the major brain regions—cortex, anterior brain stem, posterior brain stem—were determined immediately after a separate 24 hour exposure. The assay of serotonin levels is described below.

Brain serotonin turnover values as obtained by monoamine oxidase inhibition were determined as soon as possible after termination of 24, 48 and 72 hour exposures. Turnover via the probenicid method

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was measured only after a 48 hour exposure. These methods are described below.

Intravenously administered $^3$H-serotonin levels in brain, whole blood, and spleen were determined as soon as possible after termination of a 72 hour exposure. The endogenous serotonin levels in these systems were determined simultaneously. The assays for $^3$H-serotonin and endogenous serotonin are described below.

**Serotonin Level Assay.** Post-exposure brain serotonin levels were determined by modification of existing methods (Peuler and Passon, 1973; Maickel, et al., 1968). The mice were sacrificed via decapitation and brains were removed, rinsed in isotonic saline and frozen over dry ice. Each brain (or brain region) was homogenized in 4 milliliters of cold glass-distilled n-butanol in the presence of 1 gm NaCl. The homogenization was carried out in a 40-milliliter polypropylene centrifuge tube using a polytron. After centrifugation at 10,000 r.p.m. for 5 minutes at 10 C, the organic supernatant was transferred to a 13-milliliter conical glass-stoppered centrifuge tube containing 7.5 milliliters of spectro-grade n-heptane and 0.4 milliliters of 0.1N HCl/0.25 mM glutathione (Peuler and Passon, 1973). After shaking the tubes for 1 minute, the phases were separated by centrifugation (2000 r.p.m. for 2 min.) and the upper organic phase was aspirated away. Aliquots of the lower aqueous layer were transferred to reaction tubes and treated with OPT in HCl according to the method of Maickel, et al. (1968). Resulting fluorescence levels were measured with an Aminco-Bowman Spectrofluorimeter at the wavelengths of 358 mu (excitation) and 478 mu (emission). Control and
experimental determinations were performed simultaneously and compared statistically via Student's t-test described by Leone and Johnson (1965).

**Serotonin Turnover Methods.** Post-exposure brain serotonin turnover values were determined by two separate methods as described by Morot-Gaudry, et al. (1974). The first method was based on the accumulation of serotonin in the brain after administration of a monoamine oxidase inhibitor, pheniprazine (Lakeside Laboratories). This compound was dissolved in isotonic saline and injected in mice intraparatonially at 25 mg/kg. All injections were completed within one-half hour after removing the test group from the altitude chamber. At 0, 0.25, 0.50, 0.75, and 1.00 hours after injection, mice were sacrificed and brains removed, rinsed and frozen over dry ice. Serotonin levels were determined by the method described above. The slope of serotonin accumulation with time was calculated by linear regression analysis (Leone and Johnson, 1965). Control and experimental slopes (rates of turnover) were determined simultaneously and compared statistically via Student's t-test described by Leone and Johnson (1965).

The second method was based on the accumulation of 5-HIAA (the deaminated metabolite of serotonin in the brain) after administration of probenicid (Merck and Company)—an agent which blocks the active transport of such metabolites from brain cells. This drug was dissolved in 0.25 N NaOH, adjusted to pH = 7.5 with KH$_2$PO$_4$ and injected in mice intraparatonially at 400 mg/kg. All injections were performed within one-half hour post-exposure. At 0, 0.25 0.50, 0.75 and 1.00 hours after the injection, mice were sacrificed and brains
were removed, rinsed and frozen over dry ice. 5-HIAA levels were
determined by modification of a method described by Curzon and Green
(1971). Brain tissue was treated in the same manner as described
above for the determination of serotonin levels. However, the upper
organic layer (heptane/butanol) was not aspirated away but rather
transferred to another 13-milliliter conical glass-stoppered tube
containing 0.4 milliliter of 0.05M Borate/0.25 mM glutathione buffer,
pH = 10. After shaking the tubes for 1 minute, the phases were
separated by centrifugation (2000 r.p.m. for 2 minutes) and the upper
organic layer was aspirated away. Aliquots of the lower aqueous
phase were analyzed for 5-HIAA fluorescence according to the same
reaction described above for serotonin. The slope of 5-HIAA accumu­
lation with time was calculated by linear regression analysis. Con­
trol and experimental slopes (rates of turnover) were determined
simultaneously and compared statistically via Student's t-test.

3H-Serotonin Level Assay. The accumulation of 3H-serotonin
administered to mice after hypobaric exposure was determined accord­
ing to a procedure described by Lahti and Platz (1968). 3 ucuries
of 3H-serotonin (5-hydroxytryptamine-1,2-3H, 4.25 Curies per mmole,
New England Nuclear) were administered per mouse intravenously in
isotonic saline. All injections were given within one-half hour
after terminating the exposure. At one hour after the injection,
the animals were sacrificed and brains removed, rinsed, and frozen
over dry ice. Spleens and an 0.1 milliliter blood sample were also
collected and frozen over dry ice at the same time. Endogenous and
3H-serotonin were isolated and measured in all tissues according to
the method described above for brain serotonin. $^3$H-serotonin was counted in an aliquot of the final HCl/glutathione extract with 10 milliliters of Diatol scintillation fluid and a Packard Tri-Carb scintillation Counter. Control and experimental determinations were performed simultaneously and compared statistically via Student's t-test.
RESULTS

Whole brain levels after various durations of exposure (24, 48, and 72 hours) were significantly higher than controls with the greatest elevation appearing after 48 hours (Table I).

**TABLE I**

**Serotonin Levels in Whole Mouse Brain Immediately After Exposure to Hypobaric Hypoxia**

<table>
<thead>
<tr>
<th>Exposure</th>
<th>Group</th>
<th>N</th>
<th>ng/gm a</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>24 Hours</td>
<td>Control</td>
<td>6</td>
<td>572 ± 18</td>
<td>127**</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6</td>
<td>728 ± 10</td>
<td></td>
</tr>
<tr>
<td>48 Hours</td>
<td>Control</td>
<td>8</td>
<td>546 ± 15</td>
<td>138**</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>8</td>
<td>751 ± 23</td>
<td></td>
</tr>
<tr>
<td>72 Hours</td>
<td>Control</td>
<td>7</td>
<td>538 ± 12</td>
<td>122**</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>7</td>
<td>658 ± 13</td>
<td></td>
</tr>
</tbody>
</table>

Values are expressed as mean ± S.E.M. of N determinations.
**Significantly different from control, p <.01.

The time-course of the return to normality of whole brain levels after 28 hours of exposure is illustrated in figure 1. The elevation remained significant up to 16 hours after exposure and was no longer significantly different from control at 30 hours after exposure.
Figure 1. Whole brain levels of serotonin in mice at various times after exposure to hypobaric hypoxia for 28 hours. Zero (0) hour represents the time the exposed animals were removed from the stress condition. At each time interval in this figure the exposed values are significantly different than the control values (p < .05), except at 30 hours.
Serotonin levels in major regions of mouse brain immediately after 24 hours of hypobaric exposure are shown in Table II. The elevation of serotonin in the exposed animals was statistically significant in all regions examined.

**TABLE II**

**Serotonin Levels in Mouse Brain Regions Immediately After Exposure to Hypobaric Hypoxia for 24 Hours**

<table>
<thead>
<tr>
<th>Brain Region</th>
<th>Group</th>
<th>N</th>
<th>Endogenous Serotonin</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole</td>
<td>Control</td>
<td>6</td>
<td>(See Table I)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6</td>
<td>487 ± 11</td>
<td>115**</td>
</tr>
<tr>
<td>Cortex</td>
<td>Control</td>
<td>6</td>
<td>561 ± 11</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6</td>
<td>1732 ± 46</td>
<td>114**</td>
</tr>
<tr>
<td>Stem (Anterior)</td>
<td>Control</td>
<td>6</td>
<td>1515 ± 27</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6</td>
<td>2058 ± 104</td>
<td>126*</td>
</tr>
<tr>
<td>Stem (Posterior)</td>
<td>Control</td>
<td>6</td>
<td>1732 ± 46</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>6</td>
<td>2602 ± 249</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \)Values are expressed as mean ± S.E.M. of N determinations.

\( ^{*} \)Significantly different from control, p < .05.

\( ^{**} \)Significantly different from control, p < .01.
Post-exposure turnover of whole brain serotonin as estimated by the accumulation of serotonin after inhibition of monoamine oxidase is shown in Table III and illustrated in figure 2. Turnover appeared to be elevated immediately after 24, 48, and 72 hours of exposure. However, only after 48 hours was this elevation statistically significant. Turnover immediately after 48 hours of exposure as determined by the accumulation of 5-HIAA after probenicid treatment is illustrated in figure 3. Although this accumulation was slightly higher in the exposed animals, the difference from control was not statistically significant (Table III).
<table>
<thead>
<tr>
<th>Exposure</th>
<th>Group</th>
<th>Serotonin Accumulation</th>
<th>5-HIAA Accumulation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>N  ng/gm/hour(^a)</td>
<td>% Control</td>
</tr>
<tr>
<td>24 Hours</td>
<td>Control</td>
<td>18 471 ± 38</td>
<td>124</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>14 583 ± 57</td>
<td>124</td>
</tr>
<tr>
<td>48 Hours</td>
<td>Control</td>
<td>28 363 ± 31</td>
<td>138**</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>28 501 ± 43</td>
<td>138**</td>
</tr>
<tr>
<td>72 Hours</td>
<td>Control</td>
<td>15 375 ± 31</td>
<td>——</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>15 464 ± 42</td>
<td>124</td>
</tr>
</tbody>
</table>

\(^a\)Values are expressed as mean slope ± S.D. as obtained by linear regression analysis of data from Figure 2.

\(^b\)Values are expressed as mean slope ± S.D. as obtained by linear regression analysis of data from Figure 3.

**Significantly different from control, p < .02.
Figure 2. Rise in brain serotonin with time after injection of pheniprazine (25 mg/kg, i.p.). Drug was administered to mice immediately after exposure to hypobaric hypoxia for 48 hours. Lines are determined by linear regression analysis. Slopes (turnovers) are reported in Table III.
Figure 3. Rise in brain 5-HIAA with time after injection of probenecid (400 mg/kg, i.p.). Drug was administered to mice immediately after exposure to hypobaric hypoxia for 48 hours. Lines are determined by linear regression analysis. Slopes (turnovers) are reported in Table III.
The presence of intravenously administered $^3$H-serotonin in brain, spleen, and blood of mice immediately after exposure to 72 hours of high-altitude stress is shown in Table IV. Only in whole brain tissue was there a significantly increased amount of labeled serotonin relative to control levels. In the same experiment, only brain showed a significant elevation of endogenous serotonin due to the exposure.
TABLE IV

Serotonin Levels and Presence of Administered Radioactive Serotonin in Mice After Exposure to Hypobaric Hypoxia for 72 Hours. Levels of Radioactive Serotonin were Measured One Hour After Administration of Label (3 uCi $^3$H-SHT per mouse, i.v.). Label was Administered Immediately After Terminating the Exposure.

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Group</th>
<th>N</th>
<th>ng/gm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Control</th>
<th>CPM/gm&lt;sup&gt;a&lt;/sup&gt;</th>
<th>% Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brain</td>
<td>Control</td>
<td>5</td>
<td>614 ± 6</td>
<td>100</td>
<td>1834 ± 64</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>5</td>
<td>740 ± 29</td>
<td>121**</td>
<td>2456 ± 149</td>
<td>134**</td>
</tr>
<tr>
<td>Spleen</td>
<td>Control</td>
<td>5</td>
<td>8476 ± 530</td>
<td>89</td>
<td>178102 ± 16940</td>
<td>102</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>5</td>
<td>7576 ± 333</td>
<td>89</td>
<td>182305 ± 12710</td>
<td>102</td>
</tr>
<tr>
<td>Blood</td>
<td>Control</td>
<td>5</td>
<td>1574 ± 42</td>
<td>96</td>
<td>47531 ± 3254</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>Exposed</td>
<td>5</td>
<td>1508 ± 157</td>
<td>96</td>
<td>47626 ± 2674</td>
<td>100</td>
</tr>
</tbody>
</table>

<sup>a</sup>Values are expressed as mean ± S.E.M. of N determinations.

<sup>**</sup>Significantly different from control, p < .01.
DISCUSSION

The results from this study indicated that serotonin levels were increased in mouse brain tissues after exposure to chronic hypobaric hypoxia. Rutsai and Meerson (1973) reported a similar elevation in rat cerebral hemispheres but also reported a slight reduction in hypothalamic serotonin following chronic high-altitude hypoxia. Although mouse hypothalamus was not specifically isolated in the present study, the anterior brain stem which includes the hypothalamus was isolated and its serotonin was found to be elevated. It is possible that a very small reduction in hypothalamic serotonin could remain undetected when examined in this manner.

Other results from this study indicated no change in spleen and whole blood levels of serotonin after hypobaric exposure. These observations add to the list of serotonin systems which have now been examined with respect to hypoxic stress. Garcia-Perez and Lucas-Gallego (1967) found that hypoxia in the guinea pig increased lung content, decreased intestinal content and reduced excretion of serotonin. Their observations including those cited above for brain would suggest that serotonin may play a role in the adaptation of various systems to hypoxia.

The increase in brain serotonin turnover as suggested by the findings of this study appears to contradict results of a previous investigation (Davis and Carlsson, 1974) which indicated a marked reduction of turnover in rat brain. However, while the same methods
for estimating turnover were used in each study, Davis and Carlsson actually made their determinations during rather than after exposure to hypoxia. The rate of turnover in brain tissue is largely dependent on two enzymes which are responsible for the rates of serotonin synthesis and degradation—tryptophan hydroxylase and monoamine oxidase (Neff and Tozer, 1968; Morrot-Gaundry, et al., 1974). Since both these enzymes require molecular oxygen, it is possible that turnover of serotonin may be suppressed during severe hypoxia and return to normal—or possibly above normal—after hypoxia. Further, it is possible that the elevated serotonin content observed in the present study may be intimately related to the increased turnover. Hamon and Glowinski (1974) have demonstrated a slight increase in serotonin content and a marked increase in 5-HIAA content after experimentally elevating brain levels of tryptophan which are known to accelerate serotonin synthesis. Therefore, future studies should include the effects of hypobaric stress on endogenous brain tryptophan levels and the incorporation of labeled tryptophan into brain serotonin. Such investigations might describe more fully any possible relationship between serotonin content and turnover.

The increased concentration of intravenously administered $^3$H-serotonin found in mouse brain after chronic hypobaric hypoxia was a new and interesting finding. Even though each brain was rinsed thoroughly with saline prior to isolation of the labeled serotonin, it was possible that a residual amount of whole blood remains in the brain tissue and that the label present in the brains of both control and exposed animals represents blood-bound...
$^3$H-serotonin. Thus, the significantly higher level of label in the exposed animals would be a reflection of an additional amount of blood retained in their brain tissue after exposure. Theoretically this is possible due to vasodilatation (Guyton, 1971, Kogure, et al., 1970, Purves, 1972), which is known to occur in the course of adaptation to hypoxia. However, assuming this to be true, the maximum amount of additional endogenous blood serotonin would still only represent a small fraction of the endogenous whole brain increase observed in the exposed animals—i.e., approximately 3% of the total 21% endogenous serotonin increase as based on a simple calculation using blood and brain levels from Table IV.

Another explanation of the elevated $^3$H-serotonin levels is worthy of consideration. Bulat and Supek (1967) demonstrated that a significant amount of intravenously injected serotonin can move across the blood-brain barrier in normal rats—probably by passive diffusion. In a more recent study, changes in cerebral blood serotonin levels were measured in baboons during and after ischemic anoxia (Welch, et al., 1972). Serotonin levels increased in cerebral venous blood (relative to arterial blood) during anoxia and decreased shortly after the ischemic condition was relieved. The authors suggested the latter observation may be the result of increased movement of serotonin from the blood into the brain due to increased permeability of the blood-brain barrier secondary to the damaging effects of anoxia. Therefore, it would seem possible that the increased levels of both $^3$H-serotonin and endogenous serotonin
in mouse brain after hypobaric hypoxia may be the result of increased uptake from the blood stream due to an impaired blood-brain barrier. However, more conclusive evidence must be obtained before this may be considered a valid explanation of the observed phenomena.
CONCLUSIONS

Serotonin content and turnover were significantly elevated in mouse brain after exposure to chronic hypobaric hypoxia as produced by a high-altitude chamber. The elevation in content persisted for nearly one full day, post-exposure. A significantly higher level of intravenously administered $^3$H-serotonin was also found in the same tissue following the stress condition.

It was suggested that the increased content of serotonin in mouse brain may be the result of increased intracellular turnover of serotonin and/or increased uptake of serotonin from the bloodstream.
REFERENCES CITED


APPENDIX

Plates I through VI are photographs of the Altitude Simulation Unit. Plates VII through IX are schematic diagrams of systems within the Altitude Simulation Unit.
Plate I. Vacuum source illustrating the positions of the vacuum line (top left) and the exhaust line (top right) above a continuous-duty pump.
Plate II. Test chamber (side-rear view) and reference pressure apparatus (right rear).
Plate III. Test chamber (side-front view), temperature control unit (left rear), and light control switch (left front).
Plate IV. Instrument panel illustrating pressure/flow controls and readout devices.
Plate V. Test chamber (front, open-door view) illustrating chamber interior and exhaust air filter/drying unit (upper left).
Plate VI. Test chamber (rear view) illustrating lighted chamber interior as visible through the clear plastic window.
Plate VII. Mid-longitudinal section of test chamber.
Plate VIII. Schematic diagram of pneumatic system.
Plate IX. Schematic diagram of electrical system.