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## Changes in Brain Glycogen Concentration in Rats During High Altitude (12,470 ft) Exposure* (28734)

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Although the brain is more susceptible than other tissues to the deleterious effects of hypoxia and hypocapnia at moderate and high altitudes(1), few studies have investigated brain metabolism during altitude acclimatization. On the other hand, the effects on brain metabolism of acute, severe anoxia or asphyxia, *in vivo* and *in vitro*, have been frequently studied(2-12). In general, severe conditions of anoxia decreased the substrates for energy metabolism and increased metabolic end-products in brain. For example, adenosinetriphosphate, phosphocreatine, glycogen or glucose were depleted and lactic acid and inorganic phosphates were increased(2-11, see 11 for additional references). In contrast, a 24-hour fast at reduced atmospheric pressures (280 mm Hg) resulted in a 77% increase in brain glycogen in rats and guinea pigs, whereas fasting alone produced a 6% decrease(12). Also, rats acclimatized to simulated altitudes of 11,000, 18,000 and 22,000 ft for 1-3 months did not show conclusive changes in any of the preceding brain constituents, including brain glycogen(2).

The preceding studies suggest that during altitude exposure brain concentration of metabolic substrates, such as glycogen, would depend on both the severity and duration of the hypoxia. However, conditions during asphyxia and in a decompression chamber do not exactly duplicate those at high altitude. For example, during asphyxia, hypoxia is associated with hypercapnia, whereas at high altitude it is accompanied by hyperventilation-induced hypocapnia(1) and the level of carbon dioxide itself may influence brain metabolism(11). Also, in a decompression chamber, animals usually must be returned to sea level pressures in order to collect tissues for analysis(4).

In the present study, brain glycogen and plasma glucose levels were determined in rats after 3, 8, 30 and 60 days at 12,470 ft elevation and compared with values for sea level controls. Glycogen was measured because it is an important metabolic constituent of brain and because previous work from this laboratory has shown that recovery time after convulsions was prolonged at the same altitude (13), an observation which could be explained by a defect in brain carbohydrate metabolism. The latter appeared probable, because Timiras *et al*(14) have noted that glycogen concentrations in skeletal muscle, heart and liver of the rat are markedly influenced by sojourn at the same elevation.

*Methods.* The investigations were conducted on the Berkeley campus at an altitude of 250 ft (755 mm Hg) and at the Barcroft Laboratory of the White Mountain Research Station at an elevation of 12,470 ft (480 mm Hg).

Adult male Long-Evans rats with an initial body weight of about 250 g, born at sea level, were used. Conditions of nutrition (pellet diet for rats and water *ad libitum*), caging, temperature, and illumination were comparable in the animal rooms at sea level and at altitude. Animals were taken to the Barcroft Laboratory and sacrificed after 3, 8, 30 and 60 days at altitude. Sea level controls were sacrificed at ages equivalent to those of 8-day

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and 45-day acclimatized rats. Each group contained 12-15 rats. Animals were sacrificed at the same time of day, *i.e.*, 8-11 a.m. Rats were rapidly decapitated and the whole brain removed from the skull and frozen in dry ice-acetone at about −50°C. The time required for this procedure was recorded for each rat and averaged about 16 sec. The average time did not differ significantly (*t* test) between any two groups sacrificed. The frozen brain was removed from the dry ice-acetone mixture, placed on dry ice and divided into 3 parts with chilled instruments. The 3 brain areas were: forebrain (telencephalon plus diencephalon), brain stem (medulla and mesencephalon), and cerebellum. Each frozen brain section was weighed rapidly on a Roller-Smith torsion balance, then dropped into a tube of 30% KOH in a boiling water bath and broken up as fast as possible with a heavy glass stirring rod. Glycogen was precipitated with alcohol and the pellet washed three times with methanol-chloroform to remove cerebrosides and other non-glycogen constituents, according to the method of Kerr(15). Glycogen was determined as glucose by the anthrone method modified by Green and Wade(16). Blood for plasma glucose was collected from decapitated animals bled into heparinized funnels and centrifuge tubes; glucose was measured by the modified anthrone method(16).

*Results and discussion.* Means and standard errors for brain glycogen in altitude-exposed and sea level control rats are presented in Fig. 1. Glycogen levels were significantly reduced in each of the 3 brain areas—forebrain, brain stem, and cerebellum —after 3, 8 and 30 days at 12,500 ft elevation, as compared with control values. After 60 days at altitude, brain stem and cerebellum concentrations returned to normal, but forebrain glycogen was still significantly decreased.

Plasma glucose levels of rats at altitude did not differ significantly from those of the sea level controls (Table I) in this study, whereas much younger rats exhibited hypoglycemia after 3 days at altitude in an earlier study(14). It is possible that younger rats develop hypoglycemia at altitude more readily than older ones. Thus, changes in brain glycogen were not accompanied by changes in plasma glucose.

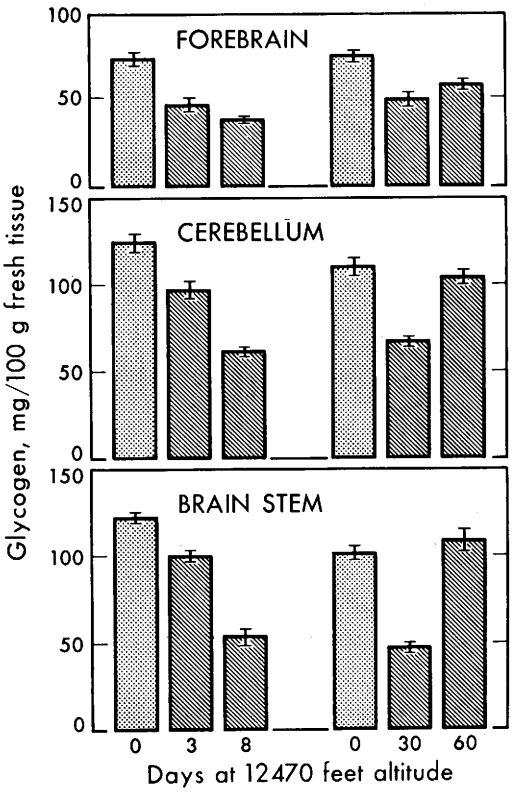


FIG. 1. Mean glycogen concentrations (mg/100 g fresh tissue) in forebrain (telencephalon and diencephalon), cerebellum and brain stem (mesencephalon and medulla) of rats after 3, 8, 30 and 60 days at 12,470 ft altitude, compared with values for sea level controls. Vertical bracketed lines are standard errors of means. Each group contained 12-15 rats.

Body weights of altitude-exposed animals tended to be lower than those of the sea level controls (Table I), in agreement with previous reports(17). Hematocrits were already significantly increased after 3 days and were increased further after 8, 30 and 60 days of altitude exposure (Table I).

Glycogen levels were significantly higher in the cerebellum and brain stem than in the forebrain of both sea level control groups (Fig. 1). Also, in the brain stem and cerebellum, glycogen was slightly, but significantly, lower in the second control group than in the first. Similarly, plasma glucose concentrations of the second control group were lower

TABLE I. Plasma Glucose Concentrations, Body Weights and Hematocrits in Rats at Altitude (12,470 Feet).

| Days at altitude | No. of rats | Body wt, g | P† | Hematocrits, % | P | Plasma glucose, mg % |
|---|---|---|---|---|---|---|
| 0 (controls) | 12 | 280 ± 8* | | 43.2 ± .5 | | 161 ± 4.0 |
| 3 | 13 | 252 ± 7 | <.02 | 47.9 ±1.7 | <.02 | 167 ± 6.6 |
| 8 | 15 | 264 ± 4 | <.01 | 51.7 ± .5 | <.01 | 159 ± 3.8 |
| 0 (controls) | 13 | 379 ±10 | | 45.3 ± .4 | | 144 ± 3.2 |
| 30 | 15 | 320 ± 4 | <.01 | 56.0 ± .8 | <.01 | 148 ± 2.2 |
| 60 | 13 | 375 ± 3 | | 56.1 ± .6 | <.01 | 142 ± 3.8 |

* Mean ± S.E.

† P values based on *t* test for significance of differences between means of values for sea level and altitude-exposed rats.

than those of the first (Table I). The reason for this is not known, but perhaps may be related to aging.

The lowered levels of brain glycogen at high altitude agree with previous reports that brain glycogen is reduced during severe hypoxia caused by breathing pure $N_2$(3,4) or 95% $N_2$ plus 5% $CO_2$(10) and by injections of sodium nitrite(5). Also, the present findings complement previous observations in this laboratory that glycogen in liver, heart and skeletal muscle is reduced after 1 and 3 days exposure to the same altitude, but returns to normal after 2 months of acclimatization(14). Glycogen levels in brain stem and cerebellum, then, are similar to levels in other body tissues in that they have returned to normal after 2 months of exposure to natural 12,500 ft elevation, whereas forebrain glycogen is still significantly reduced at this time. The failure of forebrain glycogen to normalize even after 2 months at altitude, probably indicates that this phylogenetically newer region of the brain is more susceptible to hypoxia than older areas like the brain stem and cerebellum, as has been suggested by Himwich(2, pp. 54 and 286).

Cause of the decrease in brain glycogen at altitude is not known, although it must result from a decrease in the ratio of the rates of glycogenesis to glycogenolysis. That glycogenesis may be reduced during hypoxia was shown by Prokhorova(5), who found that the specific activity of hepatic glycogen and the ratio of the specific activity of glycogen to glucose in the brain was lower in rats made hypoxic by injections of $NaNO_2$ than in control rats. Also, the transformation of labeled glucose into glycogen, fatty acids and $CO_2$ in liver slices *in vitro* was lower in samples taken from altitude-exposed rats than from sea level controls(14).

The apparent disagreement between the reduction in brain glycogen concentrations at natural 12,500 ft noted here and the lack of any effect by exposure to pressures simulating 11,000, 18,000 and 22,000 ft for 1-3 months(3) may be due to differences in techniques or procedures used. In the latter study whole brain glycogen was measured and so changes in some brain areas may have gone undetected. Also, possible effects of the sodium pentobarbital used by these workers to anesthetize the rats before sacrificing cannot be discounted.

Lowered brain glycogen concentration at 12,500 ft elevation may partially explain the significantly longer time required for rats to recover from electroshock convulsions at altitude than at sea level(13). Rapid recovery after a convulsion apparently depends on a rapid rate of metabolism, which in turn depends on the availability of oxygen and metabolic substrates(13).

*Summary.* Glycogen concentrations were significantly reduced in the forebrain, brain stem and cerebellum of rats exposed for 3, 8 and 30 days to 12,500 ft altitude, as compared with control values. After 60 days at altitude, brain stem and cerebellum glycogen concentrations returned to normal, but forebrain glycogen was still significantly decreased. The changes in brain glycogen were not accompanied by changes in plasma glucose. Hematocrit values were already significantly increased after 3 days, and increased

further after 8, 30 and 60 days of altitude exposure.

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## A Sensitive Method for Interferon Assay.* (28735)

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In cells inaccessible to some intact viruses, virus synthesis can be induced by inoculation of RNA extracted from the virus(1,2). Since the infection with viral RNA in such cells results in only one cycle of virus multiplication and since the virus synthesis can be inhibited by interferon(3,4,5) such a cell-virus system might be used for a sensitive assay of interferon. It has also been demonstrated(5) that interferon induces a high antiviral activity against poliovirus RNA in chick cells.

This report describes the effect of interferon on poliovirus RNA infection in calf kidney cells. The dose-response relationship between interferon and the inhibition of virus reproduction is analyzed and the sensitivity of the assay thus obtained is compared with other methods for titration of interferon.

*Materials and methods. Viruses.* Two plaque purified strains of poliovirus type 1 have been used, the virulent strain E 206 and the attenuated strain Lsc 2 ab. The stocks of these viruses were made in KB cells. Stock suspensions of strain 205 of Newcastle disease virus (NDV), the Sendai strain of parainfluenza virus type 1 and a strain of foot-and-mouth disease virus (FMDV) type A5 were prepared in calf kidney cells. Poliovirus and NDV were stored at +4°, the other viruses at −60°.

*Cell cultures and virus assays.* Cultures of trypsin-dispersed calf kidney cells were prepared as previously described(6). Hanks' salt solution with 0.5% lactalbumin hydrolysate (Hanks' + Lah) and 10% calf serum were used for the outgrowth and Eagle's minimum essential medium (MEM)(7) for maintenance of the cells. KB and Hela cells were prepared as described elsewhere(8).

Plaque assay of poliovirus and NDV was performed in Hela cells grown in plastic petri dishes. The overlay contained MEM, 10% horse serum and 0.95% agar (Special Agar Noble, Difco). FMDV was assayed in calf

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