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Effect of Low Atmospheric Pressure on Serum Protein of Rats

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低氣壓이 흰쥐의 血淸蛋白質에 미치는 影響

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摘 要

成熟한 Sprague-Dawley系인 雄性 흰쥐를 對照群과 實驗群으로 나누어, 對照群은 760 mmHg인 正常氣壓에 實驗群은 500 mmHg및 380 mmHg의 低氣壓에 15日間(1日當 1時間)曝露시켰다.

曝露期間中 여러 時間區에서 總 血淸蛋白質量을 Kjeldahl 및 Biuret方法에 依해 Coleman Model 295 E Spectrophotometer로서 540 nm에서 測定하였으며, 血淸蛋白質의 電氣泳動像은 Grassman-Hang (1960)方法에 依하여 barbital buffer (pH 8.6, 이온强度 0.05)로서 分離 測定하였다.

一般的으로 두 實驗群에 있어서 總 血淸蛋白質量은 對照群에 比하여 1日區에서 減少된 後, 2日區에서 부터 增加 傾向이 나타나며, 5日區에서 부터는 對照群에 比하여 높은 恒定 持續性을 나타내었고, 한편 Albumin/Globulin比에 있어서는 對照群에 比하여 1日區에서 增加를 나타내고, 그 後에는 徐徐히 減少하여 5日區에서 부터는 對照群에 比하여 낮은 恒定 持續性을 나타내었다. 特히 血淸蛋白質分劃에서 beta globulin含量이 實驗期間中 兩群 共히 有意性있는 上昇을 보였다.

一般的으로 低氣壓인 380 mmHg群은 500 mmHg群에 比하여 顯著한 變化相을 가져왔으며, 上記한 低氣壓에 曝露 5日傾부터 馴化現象을 나타내는 것으로 생각된다.

INTRODUCTION

It has become evident that the study of animal born and living in a high-altitude environment gives the most adequate approach to the understanding of how the animal body may best tolerate the constant hypoxia brought about by a decreased partial pressure of oxygen in the inspired air. In some aspects, anatomical characteristics are modified in such a way that the active tissue cells are able to receive and utilize properly the oxygen which is transported in the circulating blood at a

low tension (Hurtado, 1964).

In addition, it is important to consider that all these adaptive processes, operating in a harmonious integration, have reached in the high-altitude native resident a physiological steady state, in contrast to those found in a subject exposed to such an environment temporarily for even days and weeks (Hurtado, 1964). Consequently, it seems that the term acclimation can be applied correctly only to the high degree of tolerance which the animal exhibits the low ambient pressure.

Hurtado *et al.* (1945) reported studies on human in the Peruvian Andes in which they compared various blood values including the blood volume of normal residents at sea level with those at an altitude of 12,240 and 14,900 feet. Merino (1950) reported the changes in blood volume of six normal human adults taken from sea level to 14,900 feet, and Reynafarje (1962) reported myoglobin content and enzymatic activity of human muscle at 4,400m.

Fryers (1952) reported studies on the blood pictures of rats in 15,000 feet (427 mm Hg) and 20,000 feet (350 mmHg), Vaughan and Pace (1956), on changes in myoglobin content of rat's blood and tissues at 12,500 feet, Merrill *et al.* (1957), on the glutamic oxalacetic transaminase contents of rat's various organs and sera by exposure to a low-pressure atmosphere equivalent to 7% oxygen, Chang and Fernandez-Cano (1959), on the ovulation of rats at 410 mmHg in a low atmospheric chamber. In addition, Highman and Altland (1960) reported the serum enzyme rise after hypoxia: 20,000 and 25,000 feet (282 mmHg).

Various electrophoretic studies have revealed that the pattern of serum protein of various animals changes with altering the physiological and environmental conditions. For instance, Mayer and Heim (1960) reported that the annual hibernation in ground sqirrels is known to correspond with a reduction in the Albumin/Globulin (A/G) ratio. Experimentally, Bernasconi (1956) reported that an increase in the serum globulin has been demonstrated in the rat after the injection of anterior pituitary extracts. These and other experiments clearly demonstrate that the pattern of serum protein is quite sensitive to various physiological as well as environmental changes.

Hence, the present experiments were initiated in an effort to determine how the low atmospheric pressures (560 and 380 mmHg) influence the serum protein level and electrophoretic patterns.

MATERIALS AND METHODS

Laboratory conditioned male rats of the Sprague-Dawley strain weighing between 200~250 gm were used. Animals were divided into three groups in which one group served as the control and were maintained at normal atmospheric pressure (760 mm Hg), and the rest were subjected to one of the following two low atmospheric

pressures, 500 and 380 mmHg.

Animals were continuously subjected to these conditions for one hour a day for 15 days. A large desiccator with a capillary tube attached to a T tube, for the inlet of air, was connected to a vacuum pump and maintained at 500 or 380 mmHg. In the bottom of the desiccator, 2 liters of 1 N NaOH solution were placed to absorb the CO_2 that the animals discharged in respiration. Food and water were made available during exposure in the desiccator.

Both the low atmospheric pressure-exposed (500 mmHg) and lower atmospheric pressure-exposed (380 mmHg) rats were kept under the above mentioned conditions up to the time of sacrifice; 1, 2, 3, 5, 9 and 15 days. Blood samples were obtained with tubes from severed left saphenous vein. Serum was separated by centrifugation at 3,000 rpm for 30 minutes and kept in a refrigerator until determination of serum protein level and electrophoretic patterns. Haemolysed sera were rejected.

The total serum protein was determined at 540 nm with a Coleman Model 295 E spectrophotometer by Kjeldahl and Biuret methods. Serum protein was separated for the analysis of protein by paper electrophoresis. The determination of the value of each protein fraction was carried out by a modified Grassman-Hanning (1960) procedure in vernal buffer (pH 8.6 and ionic strength 0.05).

Three hundredth m*l* portion of each sample were applied to filter paper (Whatman No. 1) and resolved for 6 hours at 4.3 volt/cm and 0.2 mA/cm at 24±1° C. After electrophoresis, the strips were dried in an oven maintained at 100°C for 30 minutes, and dyed with ethanolic bromophenol blue. The optical density was colorimetrically determined by Toyo densitometer at a wavelength of 540 nm.

A diagram was constructed for each sample, and various fractions of the rat protein was identified by comparing it with the corresponding electrophoretic mobility of the human serum. The total serum protein levels were expressed as gram per d*l*, and each protein fraction and Albumin/Globulin (A/G) ratio as percent.

Statistical evaluation of the data was accomplished by calculating the mean and the standard deviation and A/G ratio studied. Comparision of means was made by "t" test (Croxton, 1953). All differences having a probability level of 0.05 or less were considered significant.

RESULTS

Although the general condition seemed to have deteriorated after the beginning of the experimental periods, all animals survived with no mortality.

Table 1 and Fig. 1 show the levels of the total serum protein, and Table 2 and Fig. 2 the relative proportions of the serum protein components under various conditions. Figs. 1 and 2 show the levels of total serum protein and the A/G ratio

of rats that were kept either at 500 or 380 mmHg for 15 days. Average control values were 5.75 gm/dl for the total serum protein and 1.18 for A/G ratio (Table 1 and 2).

Table 1. Total protein level in serum of rats acclimated to low atmospheric pressures of 500 and 380 mmHg.

Atmospheric pressure	Time (days) during acclimation	No. of rats	Total protein level (gm/dl)	
			Mean	Standard deviation
Control	0	10	5.75	0.40
(760 mmHg)	1	7	5.47	0.24
	2	7	5.85	0.37
	3	7	5.90	0.17
500 mmHg	5	7	6.04	0.49
	9	7	6.05	0.25
	15	6	6.00	0.37
	1	7	5.29	0.23*
	2	7	5.65	0.45
	3	7	5.96	0.54
380 mmHg	5	7	6.08	0.49
	9	6	6.09	0.49
	15	7	6.07	0.53

* $P<0.05$

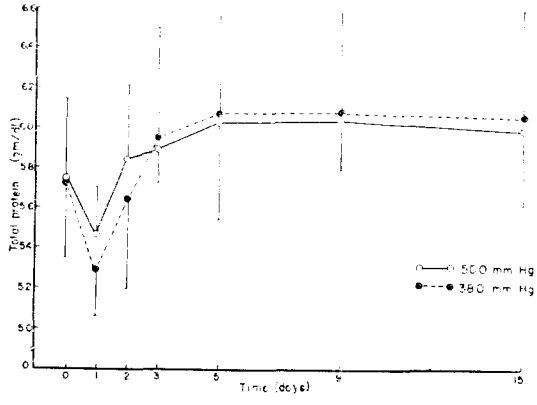

Fig. 1. Effect of low atmospheric pressures (500 and 380 mmHg) on total protein level in serum of rats at various time intervals during acclimation. Each point represents average value for the number of rats given in Table 1 and the vertical bars indicate the standard deviation of the mean.

In the total serum protein, it is clear from data that at 500 mmHg the level was decreased from 5.75 gm/dl to 5.47 during lst day, slowly increased thereafter and then from 5 days (6.04) to 15 days (6.00) expressed the higher steady state than control value. At 380 mmHg, the level during lst day was also decreased from 5.75 to 5.29, and thereafter expressed the same phenomena with that of 500 mmHg.

As shown in Table 2 and Fig. 2, in the 500 and 380 mmHg groups the A/G ratio was risen from 1.18 to 1.27 and 1.34 during lst day, slowly decreased thereafter and from 5 days (1.09 and 1.04) to 15 days (1.07 and 1.06) expressed the lower steady state than control.

In the serum protein components, specifically β-globulin was significantly increased during the experimental periods of both groups; at 500 mmHg from 16.67% to 21.18% and at 380 mmHg from 16.67% to 22.87%, respectively (Table 2).

An analysis of variance by student "t" test revealed that the effect of low atmospheric pressure on the level of the total serum protein was significant ($P<0.05$, respectively) during lst day at 380 mmHg (Table 1).

Table 2. Protein fraction and albumin/globulin ratio in serum of rats acclimated to low atmospheric pressures of 500 and 380 mmHg.

Atmospheric pressure	Time(days) during acclimation	No. of rats	Globulin (%)				Albumin (%)	A/G ratio (%)
			Gamma	Beta	Alpha 2	Alpha 1		
Control (760 mmHg)	0	10	15.80* (3.62)	16.97 (3.00)	6.53 (2.06)	6.80 (1.69)	53.90 (4.16)	1.18 (0.19)
500 mmHg	1	7	14.78 (2.97)	16.38 (1.89)	6.39 (1.51)	7.21 (1.96)	55.24 (6.23)	1.27 (0.33)
	2	7	18.77 (6.00)	17.54 (2.76)	4.84 (1.73)	5.53 (2.01)	53.32 (8.09)	1.20 (0.42)
	3	7	16.23 (3.47)	17.85 (2.71)	6.52 (1.32)	6.34 (0.46)	53.06 (1.21)	1.13 (0.05)
	5	7	15.94 (2.49)	19.23 (4.39)	6.49 (0.77)	6.35 (0.99)	51.99 (2.91)	1.09 (0.13)
	9	7	17.98 (1.34)	16.26 (1.45)	6.86 (1.68)	7.39 (1.74)	51.51 (1.39)	1.06 (0.14)
	15	6	14.58 (2.58)	21.18 (4.73)	6.26 (1.36)	6.54 (1.28)	51.44 (1.73)	1.07 (0.08)
380 mmHg	1	7	14.51 (1.98)	16.00 (0.69)	6.40 (0.47)	6.21 (0.67)	56.88 (2.20)	1.34 (0.13)
	2	7	18.69 (5.33)	17.30 (1.55)	6.10 (1.98)	5.37 (1.79)	52.54 (4.46)	1.13 (0.23)
	3	7	16.59 (3.77)	18.48 (2.42)	6.31 (1.35)	6.20 (1.40)	52.42 (2.80)	1.11 (0.12)
	5	7	15.72 (3.93)	19.48 (2.93)	7.27 (1.39)	7.14 (1.51)	50.39 (5.15)	1.04 (0.24)
	9	6	17.44 (1.44)	17.77 (1.51)	6.36 (0.70)	7.62 (1.00)	50.81 (2.20)	1.04 (0.08)
	15	7	15.15 (3.30)	22.87 (4.53)	5.78 (1.10)	5.84 (0.79)	50.96 (4.78)	1.06 (0.19)

* Mean± Standard deviation

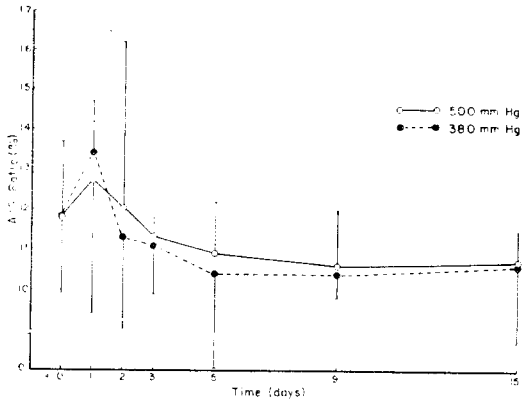

Fig. 2. Effect of low atmospheric pressures (500 and 380 mmHg) on albumin/globulin ratio in serum of rats at various time intervals during acclimation. Each point represents average value for the number of rats given in Table 2 and the vertical bars indicate the standard deviation of the mean.

DISCUSSION

It is evident in the present study that changes in low atmospheric pressure modifies the patterns of serum albumin and globulin in such a way that the Albumin/Globulin (A/G) ratio is lowered. Also, it appears that the effect of low atmospheric pressure is to alter the amount of total serum protein. In most cases the magnitude of change was higher in low atmospheric pressure-exposed rats (at 380 mmHg).

A reduction in the A/G ratio and the level of total serum protein from normal to subnormal level could be due to many types of disorders in which the albumin and globulin metabolisms were modified.

A fall in the albumin concentration may be due to an impaired synthesis or to an increased breakdown in the liver or due to the selective elimination of this fraction by kidney. On the other hand, a rise in the globulin concentration may be due to a stimulation of reticulo-endothelial system or an accumulation of abnormal protein (Abdel-Wahab *et al.*, 1956).

In other words, the variation of the A/G ratio and the level of total serum protein under various conditions is a result of many complex changes and thus hard to elucidate a single mechanism. Although the physiological significance of unexpected metabolic response is not evident to us, **it** seems to be responsible for the significant change in the serum A/G ratio and the level of the total serum protein.

One of the explanations suggested for the elevated levels of enzymes and the contents of protein in serum and muscle of animals exposed to the physiological hypoxia is a general increase in cellular permeability (Proger *et al.*, 1945; Goodale and Hackel, 1949; Vaughan and Pace, 1956; Tappan *et al.*, 1957; Highman and

Altland, 1960; Reynafarje, 1962).

Also, it has been reported that the acclimation to hypoxia influenced the circulatory and respiratory adaptive mechanisms in rats (Blood *et al.*, 1946; Fregly, 1954; Duckworth, 1961), mice (Clark, Jr., and Otis, 1952), dogs (Biddulph *et al.*, 1958; Maling and Hihgman, 1959) and various animals (Barker, 1941; Altland and Parker, 1955; Kornor, 1959). Body and organ weights during acclimation to an altitude were increased in rats (Timiras *et al.*, 1957), and the temperature regulation of mammals were affected by hypoxia (Kottke *et al.*, 1948).

The A/G ratio and the level of total serum protein were significantly lower in mice and pigeons subjected to heat stress as compared to control (Nam, 1963; Nam *et al.*, 1967), while it was higher in mice subjected to radiation exposure (Nam *et al.*, 1971).

An additional factor that may participate in regulating the level of serum protein is moderate dehydration. Yet in the present study, the elevated protein levels persisted even after water loss had been virtually replaced by drinking during the recovery period. Since the water deficit is recovered by dogs within a relatively short period (Dill *et al.*, 1933), and a constant level of plasma protein is maintained (Dill, 1938), indicating that plasma volume remains unchanged (Schmidt-Nielsen, 1964), the effect of moderate dehydration on the level of serum protein may be questioned.

The main adaptive mechanisms which intervene in acclimation during hypoxia may be generally classified into two categories; those which operate along the total pO_2 gradient from inspired air to mixed venous blood bringing about a marked economy in the drop of this gradient, so that oxygen can still pass by diffusion from blood to the active cells of the tissues, permitting the utilization of oxygen in metabolic processes; and those which are present at the tissue level, that is, an enlarged capillary bed and changes in the chemical and enzymatic processes related to internal respiration (Hurtado, 1964).

The observed increase in the activities of cellular respiratory enzymes and other biologically active substances are directed toward protecting oxidative metablism from drastic limitation in the oxygen. However, the high resistance to hypoxia observed in acclimated animals can not be explained only by the process which are directed to preserving the level of oxidative metabolism (Barbashova, 1964).

The oxidative activity of the muscle of man adapted to high altitude was generally higher than that of the sea level resident. The enzymatic change apparently occurred in mitochondrial fraction of the cell, suggesting that the respiratory enzymes are essentially involved in the process of acclimation to high altitudes (Reynafarje, 1962).

It is then logical to presume that acclimation to a low ambient pressure is also associated with compensatory processes which would facilitate the diffusion of

oxygen from blood to tissues and its proper utilization by the metabolically active cells. Such a possibility in the natives of Morococha resulted in a lower production of lactate and pyruvate than in subjects at sea level (Hurtado, 1964). Then, Hurtado (1964) indicated, somewhat paradoxically, that muscle contraction followed a more aerobic metabolic path in animals acclimated to high altitudes than in animals at sea level.

Duckworth (1961) showed that in rats acclimated to hypoxic conditions there is an adaptation at the cellular level in resting diaphragm muscle, resulting in a lowered consumption of oxygen over a wide range of oxygen pressures *in vitro*. This change is probably not due to an increase in those tissue elements with a lower respiration rate than muscle cells since histological investigation showed no apparent changes in the proportion of muscle and connective tissue elements (Duckworth, 1961).

It should be expected that in addition to the struggle for oxygen there should be a basically different type of adaptation-adjustment to hypoxia by means of a lowering of the requirement for oxygen and the use of anaerobic means for releasing energy.

The results obtained in this study support the concept that the prolonged low atmospheric pressure-exposed rats had abnormal oxidative metabolism. As the level of the total serum protein and A/G ratio of the rats express to steady state from 5 days at the low atmospheric pressures (500 and 380 mmHg), they seem to be acclimated during exposure of the above mentioned conditions.

SUMMARY

Male rats of Sprague-Dawley strain were subjected to low atmospheric pressures (500 and 380 mmHg) for an hour a day during 15 days. The serum protein patterns and the level of total serum proteins were determined at various time intervals during exposure.

1. When compared with control animals maintained at 760 mmHg, the low atmospheric pressure-exposed animal groups at 500 or 380 mmHg showed different changes in the serum protein patterns and the level of total serum proteins.

2. In the level of total serum proteins, a decrease resulted during the lst day exposure of both groups, and slowly increased thereafter. From 5 to 15 days a higher steady state value than the control was observed.

3. In A/G ratio, the sera resulted in an increase in lst day of both groups, and slowly decreased thereafter. From 5 to 15 days a lower steady state significantly than control value resulted.

4. Specifically, in the serum protein components, β-globulin was increased during the experimental periods of both groups.

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