Effects of Heat and Cold on the Serum Protein of Mice (Ⅱ)

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마우스의 血淸蛋白質에 미치는 溫熱과 寒冷의 影響에 關하여 (Ⅱ)

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摘 要

SM系인 마우스종우를 正常群과 4個의 實驗群, 即 溫熱的(33°C—60分과 38°C—60分, 每日 各各 曝露)및 寒冷的(5°C—60分과 0°C—60分, 每日 各各 曝露)群으로 나누어 最少限 22日間 馴化시킨後 濾紙 電氣泳動에 依해서 Albumin/Globulin比와 蛋白質 分劃을 測定하고 또한 血淸全蛋白質量, 血色素量, Hematocrit比, 赤血球 脆弱性및 肝臟 腎臟器官重量을 測定하였다. A/G比와 全蛋白質量은 溫熱的 增加와 寒冷的 增加의 各群이 正常群 보다 有意하게 顯著히 낮은 値를 나타내고 있다. 肝臟重量, 血色素量및 Hematocrit比는 正常群보다 溫熱的 寒冷的 條件下에서 낮은 値를 또한 나타내고 있다. 그러나 腎臟重量은 顯著하게 增加된 値를 나타내고 있다. 溫熱的 寒冷的 條件群의 肝臟에서의 組織學的 所見은 顯著한 變化는 없고 다만 血管的 變化만 나타내고 赤血球의 脆弱性은 거의 變化가 없다. 이와 같은 結果로 미루어 보아 溫熱的 寒冷的의 效果로 因해서 主로 肝臟機能 即 蛋白質代謝에 異常을 招來하는 것으로 생각된다.

INTRODUCTION

Much works have been done concerning with the effects of heat and cold in animal by Prosser *et al.* (1961), Heilbrunn (1955), Stacy *et al.* (1955) and Belehradek (1957). To date, however, there are few records concerning with the serum protein, total protein, organ weight, quantity of hemoglobin, hematocrit ratio and osmotic fragility of RBC in mice. The proteins which have been most studied from the viewpoint of physiology are blood proteins. Electrophoretic studies have revealed that the blood protein of variety of animals may be changed by altering the physiological and environmental conditions.

Mayer and Heim (1960) reported that the annual hibernation in ground squirrels corresponds with a decrease in the albumin/globulin(A/G) ratio. Experimentally, an increase in serum globulin has been demonstrated in rats and rabbits after the injection of anterior pituitary extracts by Bernasconi (1956) and Rudman *et al.* (1960). Fujiya (1961) reported that prolonged starvation and exposure to industrial wastes significantly influenced the electrophoretic pattern of several fish species. An increase in serum globulin has been studied in snake fish with osmotic pressure and electroshock stimuli by Nam and Hong (1961). Prosser *et al.* (1952) described that the relative proportion of protein components differs among different species and various pathological conditions. These experiments have demonstrated the lability of protein to physiological change but there is little information concerning with the influence of controlled external stimuli. Accordingly, this study was undertaken to determine the effects of heat, cold and day-length on the serum protein, total protein, organ weight, quantity of hemoglobin, hematocrit ratio, osmotic fragility of erythrocytes(RBC) and microscopical view of liver organ of mice.

MATERIAL AND METHODS

In this experiment, male and female mice of S.M. strain were used with weights ranging from 21g to 27g. Divided groups were placed in five conditions of heat (33°C—60 min. and 38°C—60 min. daily thermoperiod), cold (5°C—60min.

and 0°C—60 min. daily thermoperiod) and control for the period from January 2 to Feburary 2. Thus the minimal acclimation time was 22 days.

An incubator, 40×60×65 cm, was used to get dry air condition of constant high temperature held within one per cent of error. The inlet and outlet of air heat chamber were opened to protect the animal from hypoxia. No air current was allowed in the heat chamber except for natural connection. Each group of 5 mice, confined in a wire cage with floor of cork plate, were put into the heat chamber quickly. The relative humidity in the incubator was from 60 to 65% during the experiment. A refrigerator, 700×65×75 cm in size, was used to get cold air conditions of constant lower temperature held within one per cent of error. The inlet and outlet of air of cold chamber were opened to protect the animal from hypoxia. Experimental apparatus for exposing mice to cold is shown diagramatically in fig. 1. Each of 5 groups confined in a wire cage with the floor of cork plate was put into the cold chamber quickly.

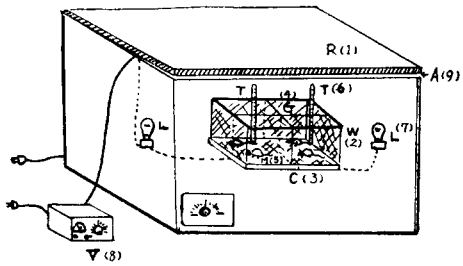


Fig. 1. Experimental apparatus for exposing mice to cold.
1) Electric refrigerator 2) Wire cage
3) Cork plate 4) Glass plate
5) Mice 6) Thermometer
7) Electric lamp 8) Voltage regulator for electric lamp
9) Air leak

Both experiments were done at laboratory room temperature from 18°C to 20°C. After acclimation, mice were starved for 24 hours and blood removed from jugular vein. The serum was separated by centrifuging at 3,000 r.p.m. for 35 minutes and either used immediately or frozen. No significant changes in blood protein were observed between fresh and frozen serum.

Paper electrophoresis was carried out by the modified Grassman-Hanning procedure(Sunderman *et al.*, 1960) in veronal buffer, pH 8.6, and ionic strength 0.05. Twenty microliter portions of serum were applied to Toyo No.51 filter paper and resolved for 13 hours at 2.5 volts/cm and 0.1 mA/cm. After electrophoresis, the strips were dyed with ethanolic bromophenol blue and optical density was determined at a wavelength of 580mμ by Toyo densitometor(type 1). Diagram of optical density was constructed comparing with the electrophoretic mobility of human serum. Total protein was determined on mice serum by Kjeldahl's and biuret methods. Organ weight was determined by a chemical balance. Quantity of hemoglobin and hematocrit ratio were determined by Hellige hemometer and hematocrit tubes, respectively. Osmotic fragility of RBC was determined by the following method. In 8 test tubes were placed, respectively, 10 ml of the following solutions made from 1% saline solution: 0.3, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60 and 0.65%. Two drops of freshly defibrinated blood were added to each tube and allowed to stand for 25 minutes and hemolysis was observed to start and complete at any concentration. The tissues from liver were obtained and were fixed in 10% formalin and stains applied were hematoxylin-eosin.

RESULTS

Diagrams of optical density revealed that there was no significant effect on sex on the serum protein of mice under the same experimental condition. The relative proportion of the serum protein components and total protein under each condition are summarized in table 1 and figure 2.

Table 1. Effects of heat and cold on serum protein of mice.

Condition	No. of mice	Total protein g%	Globulin (%)				Albumin (%)	A/G ratio (%)
			Gamma	Beta	Alpha-1	Alpha-2		
Control	15	7.3±0.3*	19.4±3.5	30.2±3.8	7.6±1.9	6.4±1.3	36.3±3.6	0.57
33°C—60 min.	15	5.8±0.3	22.5±6.3	28.9±8.2	6.7±2.4	8.3±2.0	33.8±5.8	0.52
38°C—60 min.	14	5.6±0.2	21.8±8.4	26.5±7.3	13.2±7.6	13.3±9.3	26.5±6.1	0.37
5°C—60 min.	15	5.9±0.3	20.9±4.7	27.7±5.3	12.3±6.6	8.7±3.4	31.7±8.6	0.48
0°C—60 min.	14	5.5±0.2	30.7±4.9	23.4±6.6	6.7±1.1	15.7±5.1	23.9±7.1	0.35

* Mean±standard deviation

In heat condition, it was shown that total protein and A/G ratio are less at 33°C—60 min. and 38°C—60 min. than control. This change resulted from a marked decrease in albumin and β-globulin and an increase in α-1 and γ-globulin. In cold condition, it was shown that total protein and A/G ratio are less at 5°C—60min. and 0°C—60min. than control. This change resulted from a marked decrease in albumin and β-globulin and an increase in α-1 and γ-globulin. An analysis of variance by test(Croxton, 1959) revealed that the effects of heat and cold on A/G and total protein were highly significant(the effects of heat and cold on A/G, $t=4.01>2.76$ P 0.01 and $t=3.97>2.76$ P 0.01, also the effects of heat and cold on total protein, $t=2.78>2.76$ P 0.01 and $t=2.81>2.76$ P 0.01, respectively). The relative weight of organ under each condition is summarized in table 2. In heat and cold conditions, it shows that liver organ weight is less at 33°C—60min. and 38°C—60 min. and 5°C—60min. and 0°C—60min. than control. Also kidney organ weight is greater at 33°C—60 min. and 38°C—60 min. and 5°C—60 min. and 0°C—60 min. than control. The relative values of blood components under each condition are summarized in table 3. The quantity of hemoglobin and hematocrit ratio were less at heat and cold conditions than control. There were no changes in resistance of RBC in heat, cold and control. Main microscopical finding of liver observed in the mice exposed to heat and cold was vascular change of organ without other remarkable changes. It is shown in figure 3.

Table 2. Body weight ratio for liver and kidney of mice for effects of heat and cold.

Condition	No. of mice	Mean body weight, g	Liver mean weight, g	%	Kidney mean weight, g	%
Control	15	25.00	1.37	5.40	0.20	0.80
33°C—60 min.	15	21.10	1.05	4.49	0.33	1.56
38°C—60 min.	14	25.50	1.27	4.98	0.31	1.21
5°C—60 min.	15	25.50	1.19	5.29	0.31	1.31
0°C—60 min.	14	22.22	0.92	4.14	0.27	1.21

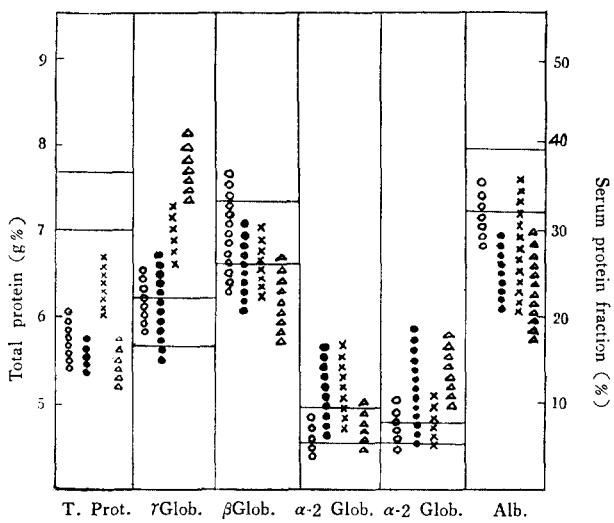


Fig. 2. Comparison of serum protein fraction in control, heat (33°C—60 min. and 38°C—60 min.) and cold(5°C—60 min. and 0°C—60 min.).

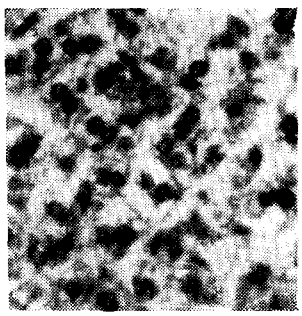
DISCUSSION

From this study, it appears that the effects of heat and cold are to alter the amount of serum total protein, protein pattern, quantity

Table 3. Effects of heat and cold on blood of mice.

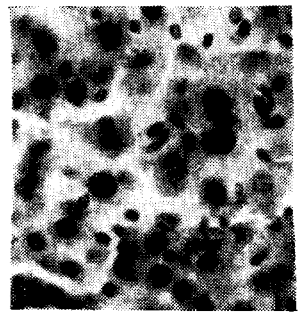
Condition	No. of mice	Hb g/dl	Hematocrit % cells	Minimum resistance NaCl %	Maximum resistance NaCl %
Control	7	14.20	41.92	0.47	0.40
33°C—60 min.	7	13.65	40.50	0.46	0.39
38°C—60 min.	6	13.10	38.52	0.45	0.40
5°C—60 min.	7	13.40	34.54	0.47	0.41
0°C—60 min.	6	13.20	34.31	0.45	0.40

of hemoglobin, hematocrit ratio and liver and kidney weights. It also shows that osmotic fragility of RBC was not remarkably changed and microscopical findings were vascular change of liver without other remarkable changes at heat and cold conditions. As the air temperature becomes lower or higher, the metabolism remains constant, while the physical insulating mechanism, thickness of body coat and vasomotor reaction, compensates for change in thermal loss. Finally



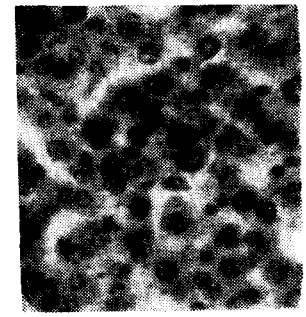
(1)

Fig. 3. (1) Normal liver of mouse. Hematoxylin-eosin stain. X430



(2)

(2) Liver of mouse(heat condition, 38° C—60 min.). Hematoxylin-eosin stain. X430



(3)

(3) Liver of mouse(cold condition, 0° C—60 min.). Hematoxylin-eosin stain. X430

these mechanisms are insufficient, and at elevated blood temperature metabolic rates rise according to tissue temperature, whereas at depressed blood temperature the thermoregulating center is stimulated to active muscular movement and probably secretion of endocrine which results in increased tissue metabolism. When the air temperature exceeds that of the skin, the body surface takes up in addition heat from its immediate surrounding: where evaporation is the only physiological way to dissipate heat. Heilbrunn *et al.* (1946) reported that heat-regulation of rodents like mice is relatively poor, at least because of poorly developed sweet gland. It was described that heat-death temperature reported for mice is at 43.3°C by Heilbrunn(1955). Numerous mechanisms of heat-death are suggested and no one mechanism operates for all animals. Heat may kill animals by enzyme inactivation, irreversible protein coagulation, toxic substances produced and change of the physical states of protoplasm by other factors. There is much species-variation in the metabolic response to low temperatures. Prosser(1961) described that when a mammal is chilled there is an increased liberation of adrenaline, increased metabolism and production of thyroid hormones, hence increased metabolism. Also it was described that adrenalectomized or thyroidectomized rats lose ability to compensate for at low temperatures by Brobeck(1946). Burton(1939) reported that thyroid activity is enhanced at low temperatures. The causes of heat- and cold-death are multiple. Heat-death often occurs at temperatures well below those of protein denaturation and cold-death needs not involve freezing. Since the syntheses of albumin and most globulin take place in the liver (Madden and Whipple, 1940), any change in liver function is likely to be accompanied by shift in the serum protein pattern and the amount of total serum protein. A study on the trout showed that the oxygen consumption of liver tissue, measured at the acclimation, was actually significantly higher at 8°C than 16°C by Evans *et al.* (1992). This metabolic over-compensation indicates that liver metabolism is greater at the low temperature. Although the physiological significance of this unexpected response is not evident to us, it is almost certainly responsible for the significant change in the serum A/G ratio, total protein, quantity of hemoglobin, organ weight and hematocrit ratio. A study showed that photoperiodism had no significant effect on the serum protein of trout (Meiser and Hickman, 1962). A seasonal modification in the serum protein pattern of mackerel has been observed, but this was attributed to difference in the nutritional state rather than maturity by Saito(1957). Rudman *et al.*(1960) reported that the pronounced effect of anterior pituitary extracts and sex hormone on the serum protein of rabbit and fowl would raise the possibility that light, by acting through the hypothalamo-hypophyseal axis to induce sexual maturity, may also influence the blood. A decrease in A/G ratio and total protein from the normal range to subnormal values is a result of many types of disease. Abdel-Wahab *et al.*(1950)

showed that a fall in albumin concentration may result from impaired synthesis or increased breakdown by the liver or from specific elimination of this fraction *via* the kidney. Also it was reported that a rise in globulin concentration may result from reticule-endothelial response or from accumulation of abnormal protein by Abdel-Wahab *et al.* (1950). Little is known about the control of blood protein concentration or of the origin and function of the various protein fraction. The variation of the A/G ratio during disease is a simple regularity superimposed on a complex pattern of change. Therefore, it may be considered that metabolism of protein is not normal state for effects of heat and cold.

ABSTRACT

Male and female mice of S.M. strain with weights ranging from 21 g to 27 g acclimated at least for 22 days to four conditions; heat(33°C—60 min. and 38°C—60 min. daily thermoperiod) and cold(5°C—60 min. and 0°C—60 min.), were examined. Serum protein was examined by electrophoresis. Total protein, liver and kidney organ weights, quantity of hemoglobin, hematocrit ratio and osmotic fragility were determined. The albumin/globulin ratio(A/G ratio) and total protein were significantly less in mice acclimated to heat (38°C—60 min.) and cold (0°C—60 min.) than in those acclimated to heat (33°C—60 min.) and cold (5°C—60 min.). However, A/G ratio and total protein of both groups were less than control. Liver weight, quantity of hemoglobin and hematocrit ratio were remarkably less in heat and cold than control. Also kidney weight was remarkably greater than control. Microscopical finding for the liver in heat and cold conditions was vascular change without remarkable other changes and osmotic fragility of erythrocyte was not remarkably changed.

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