Effect of Positive Radial Acceleration and Electroshock on the Serum Protein of Mice (1)

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마우스의 血清蛋白質에 미치는 陽性加速度와 電擊의 影響에 關하여 (1)

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摘　　要

S.M. 系인 마우스 숫우를 正常群과 4個의 實驗群 即 陽性加速度(5 G—50 秒間과 11 G—50 秒間 每日 各各 暴露) 및 電擊(5 volt—2 秒間과 10 volt 2 秒間 每日 各各 電擊)群으로 나누어 最少限 22 日間 馴化시킨 後 濾紙 電氣泳動法에 依해서 Albumin/Globulin 比와 蛋白質分劃을 測定하고 또한 血清 全蛋白質, 血色素量, Hematocrit 比, 赤血球脆弱性 및 肝, 腎臟의 重量을 測定하였다. A/G 比와 全蛋白質量은 陽性加速度의 G—force 增加와 電擊的 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 또한 正常群보다 陽性加速度, 電擊的 各條件下에서 낮은 値를 나타내고 있다. 그러나 腎臟重量은 顯著히 增加된 値를 나타낸다. 陽性加速度와 電擊的條件群의 肝臟의 組織學的 所見은 血管的 變化가 나타나고 그리고 陽性加速度에서는 또한 液胞가 出現하였다. 赤血球 脆弱性은 거의 變化가 없었다. 以上의 結果로 미루어 보와 上記 두 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

Before the use of high speed air-planes man's dealing with gravity and acceleration were ordinarily in terms of small forces and moderate changes. Postual adjustment to gravity had been evaluated chiefly in the static position. The dangers from gravitational forces in high speed air plane races were well known, but had received little systematic study. Much work have been done concerning the effect of positive radial acceleration in animal by Gell and Cranmore(1953), Stauffer(1953), Cranmore et al.(1956), Stall(1956) and Chung(1959). Electroshock often causes fetalities by accidents. It is also applied to therapy in psychiatry. Much work have been done concerning the effect of electroshock in animal by Bernad and Alpers(1942), Ferrars and Reizin(1949), Alexander(1953) and Kalinosky and Hoch(1956). To date, however, there are very few records concerning the serum protein of mice.

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 rat and rabbit anterior pituitary extracts by Bernasconi(1956) and Ruduan et al.(1960). Fujiya(1961) reported that prolonged starvation and exposure to industrial wastes significantly influenced the electrophoretic pattern of several species. Prosser et al.(1952) described that the relative proportion of protein components differs among different species and in variou pathological conditions.

Other experiments have been demonstrated the lability of protein to physiological change by Abdel-Wahab(1956), Bernasconi(1956), Sunderman(1960), Mayer(1960), Ruduan et al.(1960) and Fujiya (1961), but little information

concerning the influence of controlled external stimuli. Accordingly, this study was undertaken to determine the effect of positive radial accceleration, electroshock and day-length on the serum protein, total protein, organ weight, quantity of hemoglobin, hematocrit ratio, osmotic fragility of RBC and microscopical findings of mice liver.

MATERIAL AND METHODS

In the centrifuge runs and electroshock, male and female mice of S.M. strain were used with weights ranging from 21 g to 29 g. Divided groups were placed to five conditions: positive radial acceleration(5 g—50 sec. and 11 g—50 sec. daily periods of centrifuge run) electroshock(5 volts-2 sec. and 10 volts-2 sec. daily electroperiod) and control, from February 2 to March 2. Thus minimal acclimation time was 22 days.

An electric centrifuge was used for centrifuging the animal, Fig. 1. The centrifuge with an arm radius of 16 cm attained an acceleration of 5 g and 11 g within 7 sec. Speed of rotation was held constant within 2 percent as measured by a RPM contact tachometer. G-force was calculated from the formula. Electroshock was applied to head of mice, Fig. 2. When the electroshock was applied to mice, convulsion was induced. 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 used immediately or frozen. No significant change in blood protein was observed between fresh and frozen serum. Paper electrophoresis was carried out by a modified Grassman-Hannig procedure(Sunderman, 1960) in veronal buffer pH 8.6, ionic strength 0.05. Twenty microliter portions of serum were applied to Toyo No. 51 filter paper and resolved for 13 hours out 2.5 volts/cm and 0.1 mA/cm. After electrophoresis, the strips were dyed with ethanolic blomophenol blue and optical density was determined at wave length of 580 mμ by Toyo densitometer. Diagram of optical density was constructed and serum protein of mice identified by comparing the electrophoretic mobility to human serum. Total protein was determined on mice serum by Kjeldahl's and Biuret's 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 following method: In 10 test tubes place, respectively, 10 ml of the following solution made from the 1% saline solution; 0.3, 0.35, 0.40, 0.45, 0.50, 0.55, 0.60 and 0.65 %. Two dropes of freshly defibrinated blood were

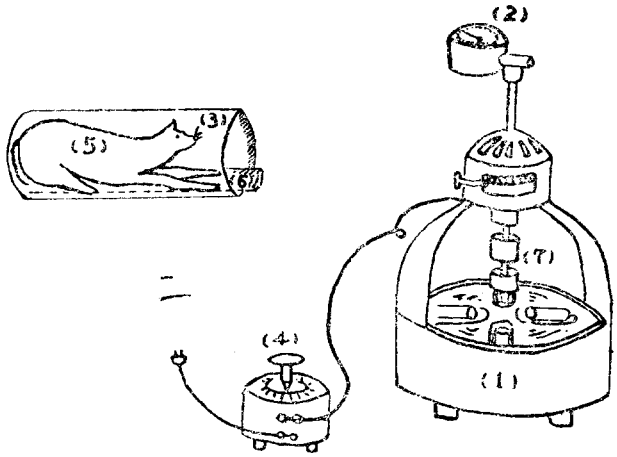


Fig. 1. Experimental apparatus for exposing mice to positive radial acceleration.
1) Centrifuge 2) Tachometer
3) Centrifuge cylinder(radius) 1.65—9 cm
4) Voltage regulator slidak' 5) Mouse
6) Iron plate for fixing 7) Mercury slip rings

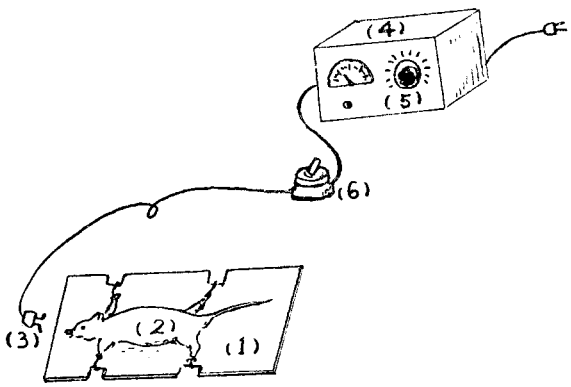


Fig. 2. Experimental apparatus of electroshock.
1) Wood plate for fixing 2) Mouse
3) Platinum stimulating electrodes 4) Voltage regulator
5) Voltmeter 6) Switch

added to each tube and allowed to stand for 25 minutes and hemolysis was observed to start and complete at any concentration. The tissue from liver was obtained and fixed in solution of 10% formalin and stains applied were hematoxylin-eosin.

RESULTS

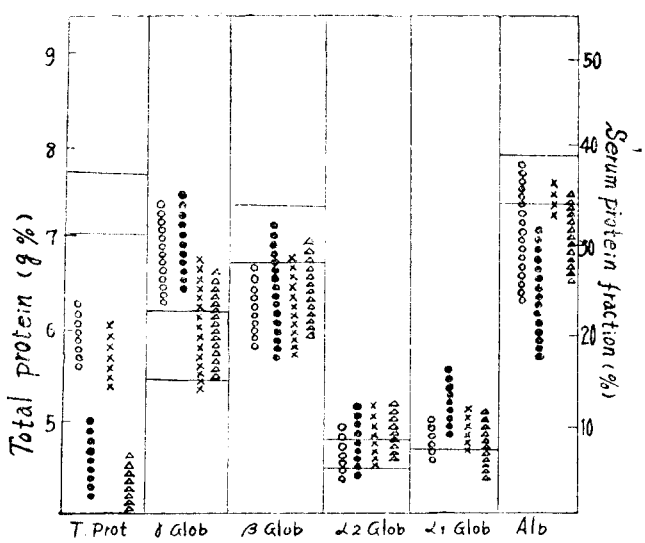
Diagrams of optical density revealed that there was no significant effect on sex for the serum protein of mice under the same experimental condition. The relative proportion of the serum protein components and total protein under each condition is summarized in Table 1 and Fig. 3.

In positive radial acceleration, it was shown that total protein and A/G ratio are less at 5 g—50 sec. and 11 g—50 sec. than control. This change resulted from a marked decrease in albumin and beta globulin and an increase in alpha-1, alpha-2 and gammaglobulin. In electroshock condition, it was shown that total protein and A/G ratio are less at 5 volts-2 sec. and 10 volts-2 sec. than control. This change resulted from a marked decrease in albumin and beta globulin and an increase in alpha-1, alpha-2 and gamma globulin. An analysis of variance by t test (Croxton 1959) revealed that the effect of positive radial acceleration and electroshock on A/G and total protein were highly significant(the effect of positive radial acceleration and electroshock on A/G $t=5.80>2.90$ $P=0.01$ and $t=3.03>2.90$ $P=0.01$. Also, the effect of positive radial acceleration and electroshock on total protein $t=6.63>2.90$ $P=0.01$ $t=5.51>2.90$ $P=0.01$ respectively. The relative weight of organ under each condition is summarized in Table 2. In positive radial acceleration and electroshock condition, it shows that weight of liver organ is less at 5 g—50 sec. and 11 g—50 sec. and 5 volts—2 sec. and 10 volt—2 sec. than control. Also weight of kidney organ is greater at 5 g—50 sec. and 11 g—50 sec. and 5 volts.—2 sec. and 10 volts—2 sec. 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 condition of positive radial acceleration and electroshock than control. There were no changes in resistances of RBC in positive radial acceleration, electroshock and control. Microscopical findings of liver observed in the mice exposed to positive radial acceleration and electroshock were vascular change of organ without other remarkable change and liver vacuolation was observed in positive radial acceleration. It is shown in Fig. 4.

TABLE 1. Effect of positive radial acceleration and electroshock on the serum protein of mice

Condition	No. of mice	Total protein g %	Globulin %				% Albumin	A/G ratio
			Gamma	Beta	Alpha-2	Alpha-1		
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
5g—50sec.	14	5.9±0.3	29.4±6.2	23.5±4.2	7.4±3.4	8.9±1.6	30.9±8.1	0.48
11g—50sec.	15	4.6±0.4	29.6±5.9	25.4±8.4	8.3±4.3	13.5±4.2	24.3±8.5	0.32
5volts—2sec.	15	5.8±0.3	21.2±7.4	23.2±5.3	9.6±3.3	10.6±2.8	35.2±2.5	0.53
10volts—2sec.	14	4.3±0.4	21.5±6.1	25.9±5.5	9.9±3.8	11.9±7.1	31.1±4.7	0.48

* Mean±standard deviation



—: Control ○: 5 g—50 sec ●: 11 g-50 sec
×: 5 volts—2 sec △: 10 volts—2 sec

Fig 3. Comparison of serum protein fraction in control, 5 g—50 sec, 11 g—50 sec, 5 volts—2 sec and 10 volts—2 sec.

DISCUSSION

From this study, it appears that the effect of positive radial accaleration and electroshock is to alter the amount of total protein of serum, protein pattern, quantity of hemoglobin, hematocrit ratio and weight of liver and kidney organs. Also, it shows that osmotic fragilities of RBC were not markedly changed and microscopical findings were vascular chage of liver without remarkable change at positive radial acceleration and electroshock condition and also liver vacuolation was observed in positive radial acceleration.

In small animals subjected to large gravitational forces in a centrifuge the lethal limits are a function of gravity(G) times(T). Under standard cnnditions, factors which influence mortality or produce physiological effects, short of death have been studied by Britton(1946). William(1956) described that guinea pigs and rats are resistant to G effects, monkeys moderately and rabbits little resistant and young animals are more susceptible than adults. No significant sexual difference was observed in the resistance of male and female mice to positive G stress by Chung(1959). In problem of protection, abdominal belts or inflated rubber bags give marked protection. William(1956) described that desoxycorticosterone offers some protection, while less is obtained from pituitary and adrenal extract, also centrifugation causes great displacement of blood to lung bases, liver and leg veins and moderate G forces of brief duration retard respiration, while longer ones produce slow, deep breathing. It was described that blood pressure is reduced immediately after moderate exposure and permanent disorientation and rigidity may occur after severe exposure by William(1956). Stacy(1955) recorded that the effect of angular accelerations and due primarily to the fact that some tissue, blood in

TABLE 2. Body weight ratio for liver and kidney of mice for effect of positive radial acceleration and electroshock

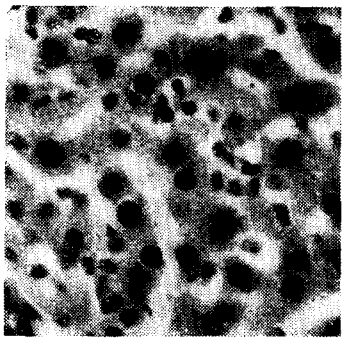
Condition	No. of mice	Mean body weight gm	Liver mean weight gm	%	Kidney mean weight gm	%
Control	15	25.00	1.37	5.40	0.20	0.80
5g—50sec.	14	23.50	0.98	4.20	0.29	1.22
11g—50sec.	15	20.00	0.96	4.60	0.31	1.55
5volts—2sec.	15	22.20	1.27	5.70	0.30	1.08
10volts—2sec.	14	22.50	0.91	4.04	0.28	1.20

TABLE 3. Effect of positive radial acceleration and electroschock on blood of mice

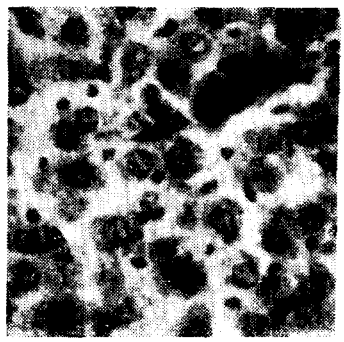
Condition	No. of mice	Hb gm/dl	Hematocrit % cells	Minimum resistance NaCl %	Maximum resistance NaCl %
Control	7	14.20	41.92	0.47	0.40
5g—50sec.	6	13.20	39.00	0.47	0.41
11g—50sec.	7	11.20	33.20	0.45	0.39
5volts—2sec.	6	13.40	39.50	0.46	0.39
10volts—2sec.	7	11.05	33.00	0.45	0.40

particular, are "loose" with respect to the remainder of the organism. It was described that the brain damage following electric convulsions is not inevitable by Bernad and Alpers(1942). Also, it was showen that the vascular reactions including hemorrhage are purely traumatic in origin and subsequently prevented by keeping the animals in good padding protection during the convulsion by Kalinosky and Hoch(1956). It was suggested that vascular reactions may be explained by excessive stimulation of the vago vasomoter centers of the medullar oblongata when the electrodes in the experimental animals are applied so that the current passes through the hind brain by Kalinosky and Hoch(1956). Physiological effects may result from the stimulation of the tissue which reacts to current, as in the case of nerve, or from the heat generated during its application. William(1956) described that in electric stimulation functional damage may occur and vital organ tissue may be impaired or destroyed.

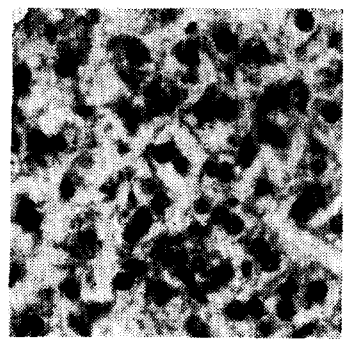
Since the synthesis of albumin and most globulin take place in the liver according to Madden and Whipple(1940),



Normal liver of a mouse, Hematoxylin-Eosin stain, 430 X



Liver of a mouse (positive radial acceleration, (11g—50sec.) Hematoxylin-Eosin stain, 430 X



Liver of a mouse (electroshock, 10volts—2sec) Hematoxylin Eosin-stain, 430 X

Fig. 4.

any change in liver function is likely to be accompanied by shift in the serum protein pattern and total protein. Although the physiological significance of these 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, hematocrit ratio and organ weight. 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. (1956) reported that a fall in albumin concentration may result from impaired synthesis or increased breakdown of the protein by the liver or from specific elimination of this fraction via kidney. Aiso, it was reported that a rise in globulin concentration may result from reticulo-endothelial response or from accumulation of abnormal protein by Abdel-Wahab et al. (1956). Little is known about the control of blood protein concentration or of the origin and function of the various protein fractions superimposed on a complex pattern of change.

SUMMARY

Male and female mice of S.M. strain with weights ranging from 21 g to 29 g acclimated for at least 22 days to four conditions: positive radial acceleration(5 g—50 seconds and 11 g—50 second- daily periods of centrifuging run) and electroshock(5 volts—2 sec. and 10 volts—2 sec. daily electro-period) were examined. Serum protein was examined by electrophoresis. Total protein, weight of liver and kidney, quantity of hemoglobin, hematocrit ratio and osmotic fragility of the red blood cell were determined. The Albumin/Globulin ratio and total protein were less in mice acclimated to positive radial acceleration(11 g—50 sec.) and electroshock(10 volts—2 sec.) than those acclimated to positive radial acceleration(5 g—50 sec.) and electroshock(5 volts—2 sec.). However, A/G ratio and total protein of both groups were less than control. Weights of liver organ, quantity of hemoglobin and hematocrit ratio were less in positive radial acceleration and electroshock than control. Also, weight of kidney was greater than control. Microscopical finding for liver in positive radial acceleration and electroshock conditions was vascular change without remarkable change and also liver vaculation was observed in positive radial acceleration. Osmotic fragility of RBC was not remarkably changed.

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