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Variation in Serum Glucose, Serum Free Fatty Acids, and Liver Glycogen Concentraions and Development of Gastric Erosions in Mice subjected to Stress

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Mice were subjected to stress consisting of restraint and water immersion and the variation in serum glucose, serum free fatty acids (FFA), and liver glycogen concentrations and the development of gastric erosions were determined at given intervals for a period of 18 hr.

In control mice which were deprived of food and drinking water, no significant variation was observed in serum glucose and FFA levels throughout the experimental period. In contrast, their liver glycogen content was virtually exhausted 9—12 hr after fasting and then began to accumulate, resuming its 6-hr value 18—24 hr after fasting. In stressed mice, serum glucose levels decreased after 1—3 hr of stress and increased thereafter, showing a significant elevation in the levels compared with its 3-hr value 18 hr after stress. On the contrary, FFA levels increased for the initial 1—3-hr period, declined 12 hr after stress, and showed a significant decrease compared with the corresponding value in the control group 18 hr after stress. Liver glycogen was practically exhausted for the first 12-hr period after stress. Thereafter, its levels increased but was still lower than those in the control group 18 hr after stress. Gastric erosions were generated after 1 hr of stress and developed with the progress of time, reaching the highest severity of erosions at the end of stress.

It has been reported that gastric erosions which can be quantitatively estimated develop in mice subjected to restraint and water immersion[2,3] and that this stress model can be applied for screening of drugs.[3] Erosions induced in this way are recognized as an acute vital response of the body to stress. Furthermore, such vital response is not limited to erosions, but is revealed as a lot of physiological changes. For example, various body components in the rat are influenced under stress conditions such as forced running[4,5] or cold exposure.[6,7] And the rat is the first choice among small animals in such kinds of studies. To date, no study on changes in body components in mice subjected to so severe stress condition as to develop gastric erosions has been reported. In the present study, in order to know the influence of stress on mice, the variation in the concentrations of serum glucose, serum free fatty acids (FFA), as well as liver glycogen, and the development of gastric erosions were examined in mice subjected to restraint and water immersion, at given intervals.

Experimental

Method——Male ddys strain mice weighing 19—21 g were used. The short term experiment and the long term experiment were carried out on mice subjected to stress according to the method of Yano, *et al.*,[3]

1) Location: *a) Yayoi-cho, Chiba, 280, Japan; b) Inohana-cho, Chiba, 280, Japan.*
2) C.J. Pfeiffer, "Peptic Ulcer," ed. by C.J. Pfeiffer, J.B. Lippincott Company, Philadelphia, 1972, p. 84.
3) S. Yano and M. Harada, *Japan. J. Pharmacol.*, **23**, 57 (1973).
4) P.D. Gollnick, P.G. Soule, A.W. Taylor, C. Williams, and C.D. Ianuzzo, *Am. J. Physiol.*, **219**, 729 (1970).
5) K.M. Baldwin, J.S. Reitman, R.L. Terjung, W.W. Winder, and J.O. Holloszy, *Am. J. Physiol.*, **225**, 1045 (1973).
6) Y. Hashimoto, T. Nishimura, Y. Kurobe, Y. Kohashi, M. Kakie, and J. Ando, *Japan. J. Pharmacol.*, **20**, 441 (1970).
7) G.J. Klain and J.P. Hannon, *Federation Proc.*, **28**, 965 (1969).

in which animals were immobilized in the stress cage and immersed in the water bath of 25° to the depth of the xiphoid.

In the short term experiment, animals were deprived of food at 10 a.m., subjected to stress at 7 a.m. on the next day, and sacrificed at 1, 2, and 3 hr after stress respectively. In the long term experiment, mice were deprived of food at 10 a.m., subjected to stress at 4 p.m. on the same day, and sacrificed at 3, 6, 12, and 18 hr after stress respectively. Concerning mice served as control, they were deprived of food at the same time as the stressed group and were deprived of drinking water at the time of the onset of stress. All mice except during the period of stress were kept in a room of 22° and 60% humidity until sacrifice.

At the end of stress, animals were freed from the cage and blood was taken by means of cardiac puncture under ether anesthesia. Blood from 3 mice was pooled to make one blood sample and serum was prepared by centrifugation. The serum was diluted double with saline for determination of serum glucose and FFA. One mouse was arbitrarily selected out of 3 mice whose blood was pooled and its liver was excised at the time of death, frozen immediately, and stored in a freezer (−15°). The stomach of all animals was isolated and fixed with formalin for estimation of gastric erosion. Serum glucose concentrations were measured by Sasaki's method[8] which modified the *o*-toluidine technique established by Hyvärinen, *et al.*[9]; 0.05 ml samples of the diluted serum and Diagnotesta-G (Daiichi kagaku yakuhin) were used. FFA concentrations were determined by Dole's method[10]; 1.0 ml samples of the diluted serum were used. After 0.5 g of the liver was digested in 30% KOH, its glycogen contents were measured with anthrone reagent by the indirect method described by Seifter, *et al.*[11] The severity of gastric erosions (erosion index (EI)) was estimated according to the method of Yano, *et al.*[3] Statistical analysis of data was done according to the Student's *t* test and significant differences were given at the level of 5% or less than 5%.

Results

All of the results is presented in Fig. 1.

1. The Short Term Experiment

Serum glucose concentration in the control group maintained approximately constant levels of 86—99 mg%, while that in the stressed group decreased 1 hr after stress and levels of 57—61 mg% were sustained for 2 hr thereafter. FFA concentration in the control group showed approximately constant levels of 1.28—1.49 mEq/liter. On the other hand, FFA concentration in the stressed group increased up to 2.13 mEq/liter 1 and 2 hr after stress and still remained at an increased level compared with the corresponding value in the control group 3 hr after stress. Liver glycogen content in the control group ranged from 4.2 to 7.1 mg/g wet wt (expressed as mg/g below), while that in the stressed group decreased to such extreme low levels as 0.3—0.4 mg/g 1—3 hr after stress. Gastric erosions were generated 1 hr after stress and the 3-hr value of EI amounted to 4.8.

2. The Long Term Experiment

Serum glucose concentration in the control group showed a tendency of a progressive decrease ranging from 129 to 92 mg% but even the largest difference between the highest and the lowest value was not significant. In contrast, serum glucose concentration in the stressed group decreased to a value of 82 mg% 3 hr after stress and its decrease turned into a progressive increase thereafter. It reached an increased level of 209 mg% 18 hr after stress with a significant difference from the decreased level of its 3-hr value. In another series of the experiment, the 18-hr value of serum glucose concentration in the stressed group was significantly higher (168 ± 17 mg% ($n=8$), $p<0.05$) than the corresponding value in the control group (122 ± 9 mg % ($n=8$)).

FFA concentration in the control group showed approximately constant levels of 1.02—1.17 mEq/liter during the whole experimental period. In the stressed group, a significant increase in FFA concentration which went up to 1.28 mEq/liter was observed 3 hr after stress

8) M. Sasaki, *Rinsho Byori*, **12**, 434 (1964).
9) A. Hyvärinen and E.A. Nikkilä, *Clin. Chim. Acta*, **7**, 140 (1962).
10) V.P. Dole, *J. Clin. Invest.*, **35**, 150 (1956).
11) S. Seifter, S. Dayton, B. Novic, and E. Muntwyler, *Arch. Biochem.*, **25**, 191 (1950).

and this increased level declined to 0.92 mEq/liter 12 hr after stress. FFA concentration 12 hr after stress and 18-hr after stress decreased significantly compared to the 3-hr value in the stressed group and to the 18 hr value in the control group respectively. In another series of the experiment, the 18-hr value of FFA levels in the control group was 1.17±0.07 mEq/liter (n=8), which was significantly different from that in the stressed group (0.88± 0.06 mEq/liter (n=8), $p<0.01$).

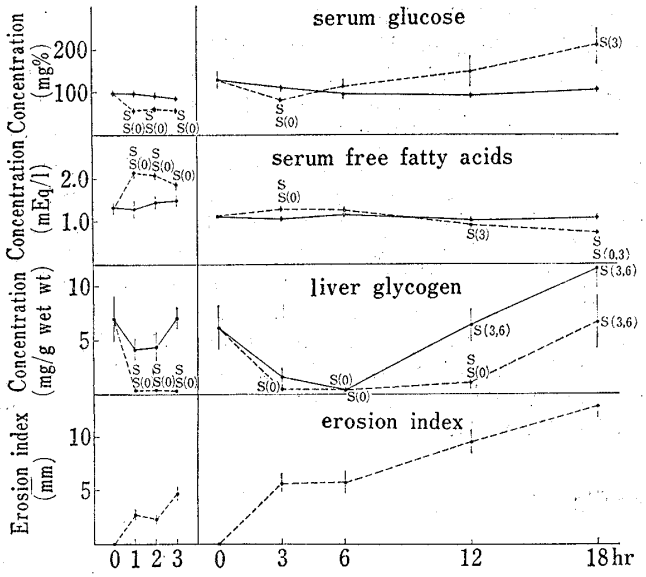

Fig. 1. Time Course of the Variation in Serum Glucose, Serum Free Fatty Acids, and Liver Glycogen and of the Development of Gastric Erosions in the Short Term Experiment (left) and the Long Term Experiment (right)

Stress was initiated at 0 hr. A full line and a dotted line refer to the control group and the stressed group, respectively. Each point is given as the mean ±S.E. of 8 experiments in the determination of serum glucose, serum free fatty acids, and liver glycogen and of 24 experiments in the estimation of gastric erosions. S in the figure indicates that the value in the stressed group was significantly different at $p<0.05$ from that in the control group. S (0, 3, or 6) indicates that the value in the stressed group or the control group was significantly different at $p<0.05$ from that of its own group at 0, 3, or 6 hr, respectively.

Liver glycogen content in the control group decreased during the first 6-hr period and its 6-hr value was substantially zero (from 6.1 to 0.4 mg/g). Thereafter, its levels began to increase, recovered to the original one 18-hr after fasting (6.3 mg/g) and showed a tendency of a further increase. In the stressed group, on the other hand, liver glycogen was almost exhausted similarly to the control group during the first 6-hr period and these decreased levels were maintained during the next 6-hr period, having a significant decrease compared with the corresponding levels in the control group. In the last 6-hr period its decreased levels began to increase, reaching the original levels 18 hr after stress (6.6 mg/g). In another series of the experiment, the 18-hr value of liver glycogen content was still low (2.8±0.9 mg/g (n=8), $p<0.01$), showing a significant decrease compared with the corresponding value in the control group (8.8±1.7 mg/g (n=8)).

Gastric erosions developed with the progress of time, eliciting the highest EI of 12.9 18 hr after stress. No gastric erosions were generated in the control group.

Discussion

It is necessary to take account of the influence of fasting and deprivation of drinking water on mice, since mice are not accessible to food and water during a period of stress. In both short and long term experiments, serum glucose levels showed no significant difference from its original level at any given time throughout the period of fasting and water deprivation. Müller, *et al.*[12] measured blood glucose of Swiss Webster male mice enzymatically and demonstrated that an original value of blood glucose concentration (215 mg%) decreased under fasting and reached an approximately constant level (70 mg%) 40—64 hr after fasting. Increase in FFA levels under fasting is known[13] but FFA levels in the present two experiments were almost constant through the whole experimental period, which suggests no or slight, if at all, influence of fasting and water-deprivation on the concentration of FFA. Another

12) E.E. Müller, D. Miedico, G. Ginstina, and D. Cocchi, *Endocrinol.*, **88**, 345 (1971).
13) H.A. Harper, "Review of Physiological Chemistry," 14th ed., Maruzen Asian Edition, Maruzen Company, Tokyo, 1973, p. 290.

well-known fact is that liver glycogen content decreases markedly under fasting. Since liver glycogen content in mice which were allowed free access to food and water amounted 36.9±5.1 mg/g (n=8) in the present experiment, it was found that liver glycogen content had already decreased considerably at the time of the onset of stress, *i.e.*, 6 hr after fasting in the long term experiment. Further, liver glycogen content continued to decrease so extremely that its value was almost zero after fasting of 9—12 hr. However, such low levels of liver glycogen were not sustained but elevated 18 hr after fasting and returned to a level of the 6-hr value thereafter. This recovered level nearly corresponded to the levels observed in the short term experiment. These findings suggest that gluconeogenesis was accelerated by itself or as summation of it and a decrease in glycogenolysis because of maintenance of fasting and that an increase in liver glycogen content resulted.

The restraint and water immersion stress progressively increased gastric erosions in mice and influenced serum glucose, FFA, and liver glycogen concentrations in the following manner: a decrease at the initial stages and an increase at the late stages in serum glucose levels, an increase at the initial stages and a decrease at the late stages in FFA levels, and a practical exhaustion at the initial stages as well as middle stages and a gradual accumulation at the late stages in liver glycogen content. Under such a stress condition, animals are highly influenced by struggle (movement) and shivering which require more energy and by heat loss to surrounding bath water at the initial stages, by heat loss during the period when struggle has markedly disappeared, and by blood loss from the digestive tract (mainly from the stomach) during the period when erosions are developing.

Gollnick, *et al.*[4] subjected rats to running exercise and found a decrease in blood glucose and liver glycogen levels and an increase in plasma FFA levels. Gastrocnemius muscle glycogen content was also reduced. They concluded that a release of catecholamines from the adrenals was not essential for controlling glycogenolysis and that both adrenergic as well as nonadrenergic control operated in the adipokinetic response. Baldwin, *et al.*[5] also subjected rats to running exercise and observed a slight decrease in the concentration of blood glucose preceded by its initial moderate increase, a marked increase in FFA levels, and a decrease in liver glycogen concentration, discussing a more important role of liver glycogen as an energy source than that of muscle glycogen. Hanson, *et al.*[14] observed plasma FFA levels in man increasing under cold exposure and concluded this change might be produced through several possible mechanisms the clearest of which was perhaps seen in the action of noradrenaline. Exposing rats to a cold environment, Hashimoto, *et al.*[6] found that plasma glucose concentration increased at the initial stages and resumed the original level thereafter and that plasma FFA concentration maintained marked increased levels, and they discussed the necessity of both plasma glucose and FFA for maintenance of body temperature.

Under cold exposure of animals, a release of catecholamines from the adrenals and sympathetic nerve endings is augmented and their excretion in urine is also enhanced. We have observed a marked increase in excretion of catecholamines (mainly adrenaline) in urine during the initial 3-hr period in rats subjected to the restraint and water immersion (25°) stress (unpublished data). Klain, *et al.*[7] reported enhanced activities of enzymes such as glutamic pyruvic and glutamic oxaloacetic transaminases, glucose-6- and fructose-1,6-phosphatases, and phosphoenolpyruvate carboxykinase which are associated with gluconeogenesis, 2 days after exposure of rats to cold environment. Oyama, *et al.*[15] subjected mature female rats to centrifugation procedure and observed an acceleration in liver glycogenesis which was always preceded by an elevation in total synthetase activity due to an increase in the glucose-6-phosphate independent form of the enzyme.

The time course of the variation in serum glucose, FFA, and liver glycogen concentrations, and the development of EI in the present study may be summarized as follows. At the initial

14) P.G. Hanson and R.E. Johnson, *J. Appl. Physiol.*, **20**, 56 (1965).
15) J. Oyama and B.C. Daligcon, *Endocrinol.*, **80**, 707 (1967).

stages of stress, consumption of serum glucose and FFA was increased because of continuation of struggle and shivering and of maintenance of body temperature, glycogenolysis and lipolysis were accelerated, liver glycogen content was virtually exhausted, serum glucose levels decreased and FFA levels increased. It seems that an augmentation in sympathetic nerve activity, an increase in a release of catecholamines from the adrenals as well as an increase in adrenocorticotropic hormone (ACTH) secretion, and other factors participated in such a variation in these body components. As stress progressed, gluconeogenesis began to be accelerated, which probably resulted in an increase in serum glucose levels together with a decrease in FFA levels 12 hr after stress and in a further increase in serum glucose concentration accompanied with a further decrease in FFA concentration and with accumulation in liver glycogen content 18 hr after stress. Glucocorticoids might play a role in these changes. However, problem of suppression of glycogenolysis remained in this case. Gastric erosions were generated 1 hr after stress and EI progressively developed.

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In Vitro Binding of Sulfobromophthalein to Cytoplasmic Protein from Liver, Kidney, and Small Intestinal Mucosa of Rat and Rabbit[1)]

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Sephadex G-75 gel filtration of cytoplasmic protein with BSP *in vitro* yielded elution patterns characteristic of the protein sources as liver, kidney, and small intestinal mucosa of rat and rabbit. In the both species, binding of sulfobromophthalein (BSP) with Y protein was observed to be predominant in the liver, and weak or not recognized in the kidney and small intestinal mucosa, as was to be expected. On the other hand, the BSP elution with Z protein fraction was appreciable in the rabbit kidney and the rat small intestinal mucosa, and it appeared that the role of Z protein on the fate of the organic anion in the body was not so simple. Two unknown fractions, Y′ and Y″ protein, were recognized in the BSP elution pattern with cytoplasmic protein of the rat kidney, and Y′ protein was recognized in that of the rabbit kidney and small intestinal mucosa.

Sulfobromophthalein (BSP) is used clinically in the testing of hepatic function because the normal liver excretes most of the dye within a short time. To elucidate the mechanism of the hepato-biliary excretion, the binding of BSP to the rat liver has been studied by several research groups in recent years. Arias, *et al.*[3–7)] studied the roles of the rat liver cytoplasmic

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7) G. Fleischner, J. Robbins, and I.M. Arias, *J. Clin. Invest.*, **51**, 677 (1972).