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Amino-acid metabolism related to glucose infusion in endotoxic shock

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In endotoxic shock provoked by giving endotoxin in dogs, amino-acid metabolism was evaluated in relation to hemodynamic changes and glucose utilization. It is defined that endotoxic shock causes liver dysfunction and a decrease in portal blood flow with induced metabolic acidosis.

On the basis of a study of amino-acid metabolism, it is of interest to emphasize that the salient results in endotoxic shock in dogs are promoting release of ALA and PHE from the skeleton muscle and increasing uptake of LEU, ALA and GLY into the liver. In conclusion, the hyperosmolar glucose infusion did not offer any advantage over the isotonic or restricted glucose one.

INTRODUCTION

The report concerning circulatory collapse caused by serious infection was first noted in 1831 according to literatures. Since then, it has become well known that shock caused by serious infection was more often seen in bacteriemia, in particular the concept of endotoxic shock derived from gram-negative bacteria was enunciated by WAISBRER¹⁾ in 1951. It has also been confirmed by BOIVIN²⁾ in 1933 that the nature of endotoxin is composing of lipopolysaccharide.³⁾⁻⁵⁾ Since Limulus test by LEVIN and BANG⁶⁾ in 1960 was clinically used for detecting endotoxin, it has been popularized and in widely clinical use. The study on the pathogenesis of endotoxin itself was progressed in the research fields of circulation, metabolism and blood coagulation.⁷⁾⁻⁹⁾ It is well known that endotoxic shock produced direct or indirect metabolic arrangement due to either direct cytotoxic acti of endotoxin or indirect peripheral circulatory failure by resulting vasospasm and increasing permeability of vessel walls. And it is agreed in general that endotoxin inhibits O₂ consumption and ATP production of mitochondria¹⁰⁾ subsequent to disturbance of homeostasis, accompanying

abnormal responses of the autonomic nerve and the endocrine systems and circulatory failure. On the other hand, it is noted that glyconeogenesis is impaired enough to lead to hypoglycemia with respect to glucose metabolism.¹¹⁾ In addition, it is recognized that glucose uptake and utilization at the periphery in endotoxic shock are much depressed enough to bring the paucity in energy production,¹²⁾⁻¹⁴⁾ compensated for by oxidation of aminoacid in the muscular tissues although WICHTERMAN *et al.*¹⁵⁾ report that glucose utilization at the periphery is enhanced with an aid of accelerated insulin action in sepsis. DUFF¹⁶⁾ and DANIEL¹⁷⁾ clarified that paucity of energy production in endotoxic shock was possible to be compensated for by oxidation of amino-acids derived endogenously.

The purpose of this study is to ascertain as to whether glucose administration in endotoxic shock is effectively compensated for paucity of energy production or not.

MATERIAL AND METHOD

Mongrel dogs weighing between 7~24kg with an average of 13kg were used for this study under a control of fasting on preceding night and anesthetized with intravenously administered pentobarbital sodium (30mg/kg) and maintained on positive pressure ventilation (15ml/kg in tidal volume, 16 to 18/min in respiratory rate). The catheters for monitoring the pressures and taking blood samples were introduced into the femoral artery and vein, portal vein and hepatic vein.

The blood flow of the portal vein was measured with the probes of 5 to 8mm in outside-diameter using the electromagnetic flow meter (MVF-2100 Nihon Koden).

At the stable circulatory state, sampling and monitoring were made to gain the values as a control before induction of endotoxic shock which was induced by bolous injection of 3mg/kg of endotoxin (LPS *E. coli* O26 B6). The levels of the blood sugar (by glucose oxidase method), lactate, pyruvate (by enzymatic method), insulin in plasma (by RIA method) and GOT, GOP (by Wroblewski-Karmen method) as well as blood gas analysis with use of Astrup analyser were measured. The animals used in this study were divided according to fluid transfusion, EX group included 10 dogs in whom 5ml/kg/h of saline was given and EX+G group comprised 6 dogs in whom 0.5g/kg/k of hyperosmolar glucose was administered. The levels of amino-acid were measured using the amino-acid analyzer (JLC 200A Nihon Koden) from the blood samples which included the femoral artery and vein, portal vein and hepatic vein and also the surgical specimens obtained from quickly excised part of the left lobe of the liver.

These samples were centrifuged at 4°C for 15min. The plasma were pretreated with 10% tri-chloroacetic acid for deproteination. Tissue amino-acid levels were measured from the biopsied tissues obtained from parts of the lower margin of right lobe of the liver and the quadriceps femoris muscles. According to extration method applied for tumor-contain-

ing antigen, exactly one gram of liver tissues was homogenized with 0.01m phosphor-buffer saline (pH 7.3 at 23°C) using a homogenizer (IKA WERK). Equal amount of 10% trichloroacetic acid were added to supernatant for deproteination.

Data were expressed as means+SEMs, Student's t-test was used for statistical analysis and P-values of less than 0.05 were considered to be significant.

RESULTS

Systemic prssure and portal blood flow (Fig 1, 2): Mean arterial pressur (MAP) rapidly decreased after giving endotoxin, LPS. A nadir systemic pressure was 59.4 ± 26 mmHg in EX group and 45.7 ± 4 mmHg in EX+G group. Since then, they returned to the values before giving endotoxin and at 30min they kept 75% in EX group and 70.9% in EX+G group as compared with the initial levels. Thereafter, they gradually swifited descent and at 3 hours reduced by 35.7% and 45.9% respectively. There was no statistical significance between both groups. Changes in the portal blood flow were almost similar in spite of a marked reduction immediately after LPS administration.

Blood gas analysis (Fig. 3): PH in the blood of th femoral artery gradually reduced. At 30min it showed 7.30 ± 0.09 in EX group and 7.31 ± 0.09 in EX+G one with statistical significance, thereafter it continued to decrease more and more. The levels of HCO_3 and BE showed the same as PH, although Po_2 and PCO_2 levels did not significantly vary.

Changes in GOT and GPT (Fig. 4): The values of GOT and GPT increased at 3 hours. The GOT values reached 144.7IU/I in EX group and 216 IU/I in EX+G one with remarkable change although GPT showed 44.3IU/I and 61.8IU/I respectively. Changs in

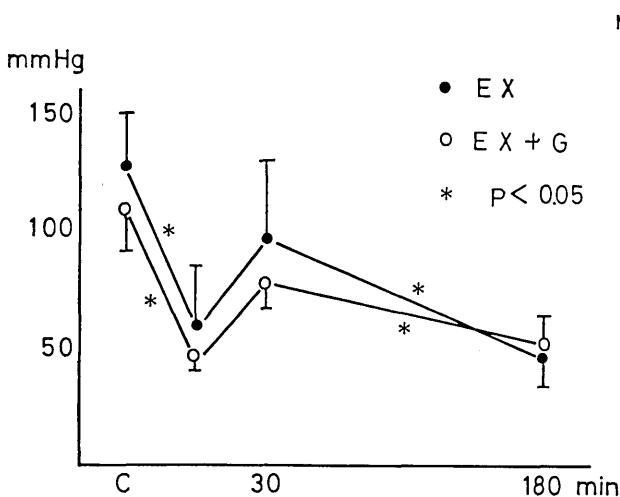


Fig. 1. Mean arterial pressure

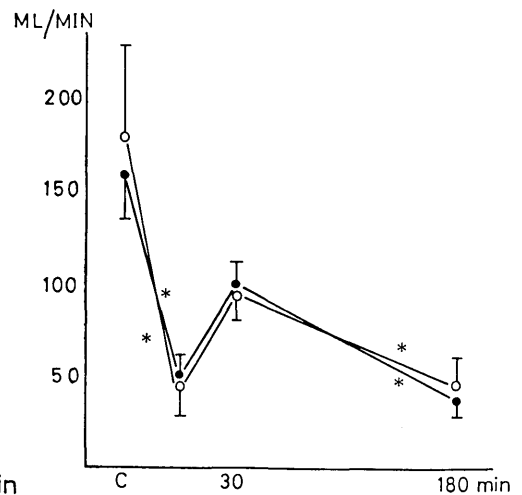


Fig. 2. Portal blood flow

lactate and pyruvate (Fig. 5). The lactate levels increased 44mg/dl in EX group at 30min and 37.4mg/dl in EX+G one, the pyruvate one showing a similar tendency. Excess lactate calculated indicated a significant increase at 30min. At 3 hours it was the same as that at 30min with significant difference between both of them.

Changes in blood sugar and immunoreactive insuline (IRI) (Fig 6): The blood sugar values somewhat increased in EX group at 30min in spite of showing a marked reduction (48.5mg/dl) although these remarkable rose in EX+G group anreached 441mg/dl at 3 hours. IRI levels were raised, showing heperinsulinemia as being 21m μ g/dl at 30 min, thereafter those were reduced to 10m μ g/dl. In EX+G group, IRI values correlated with blood sugar levels and increased to 180.8m μ g/dl at 3 hours.

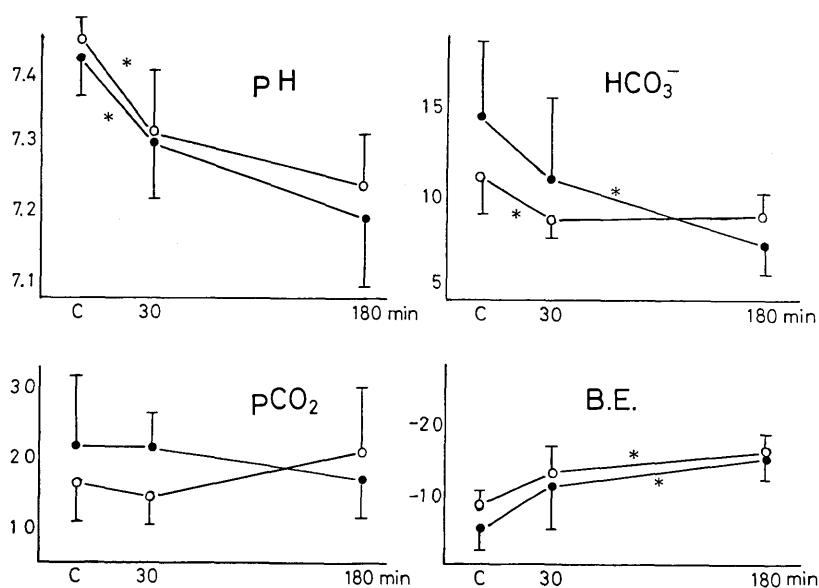


Fig. 3. Blood gas

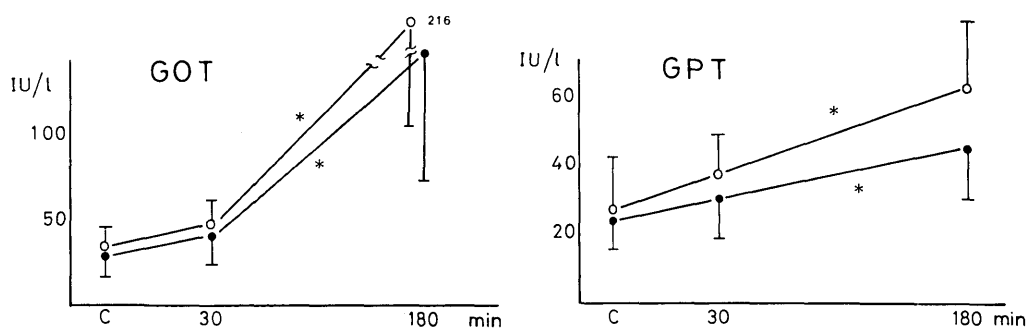


Fig. 4. Liver function

Free amino-acid level variation (Fig. 7, 8, 9): Free aminoacid levels were compared among the blood samples taken from the femoral artery and vein, portal vein and hepatic vein. ALA in EX group increase in all the blood samples. GLY levels were uniformly high, in particular, a significant rise in GLY in the portal vein was noted at 3 hours. Judewuse, BCAA levels rose up except in the femoral vein.

Changes in PHE also were similar with a significant difference in the femoral vein at 3 hours. On the other hand, variation of BCAA concentration in EX+G group were different from those in EX one and tended to be reduced.

Difference in free-aminoacid and blood sugar between femoral artery and vein (Fig, 8, 9, 10): The release of ALA was marked in both groups. And also uptake of LEU, one of BCAA, was significant in EX group at 3 hours. PHE was gradually released and it has become significant at 3 hours. Changes in blood sugar levels showed a tendency toward precipitating the incorporation.

Tissue amino-acid in skeleton muscle (Fig 11): The ALA levels were significantly raised at 3 hours in EX group and at 30min in EX+G one. GLY values markedly increas-

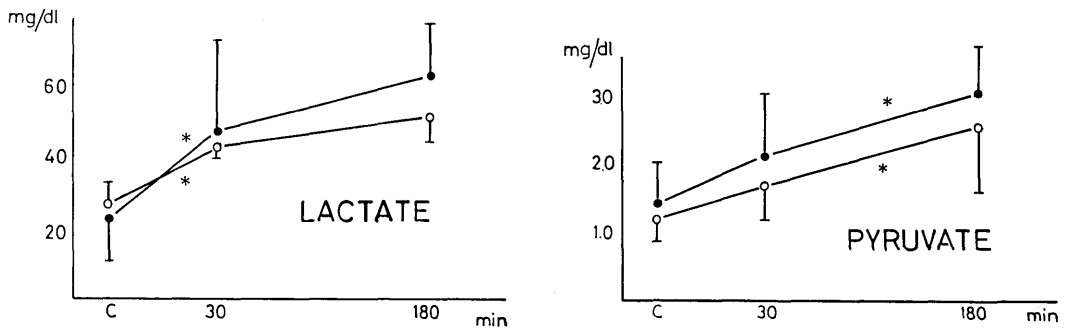


Fig. 5. Lactate and Pyruvate Excess lactate
 EX: 30min 19.6±17.3 180min 19.6±8.9
 EX G: 30min 15.4± 7.7 180min 14.9±9.0

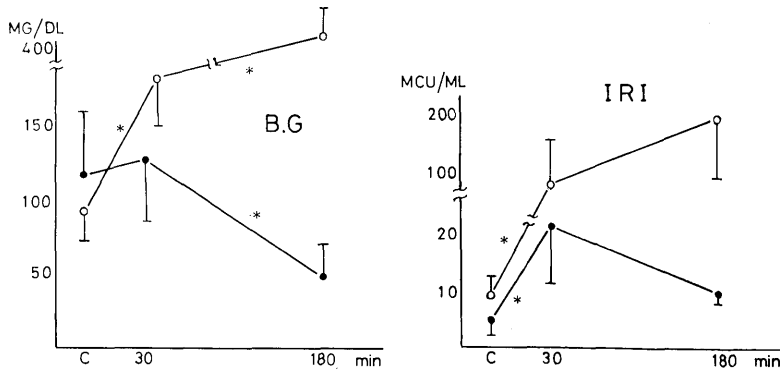


Fig. 6. Blood glucose and immunoreactive insulin

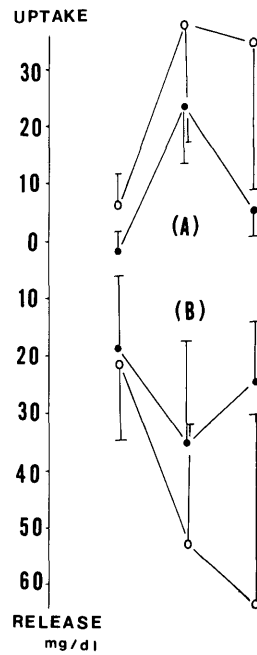


Fig. 7. (A) Femoral arterio-venous differences of blood glucose
(B) Porto-hepatic venous differences of blood glucose

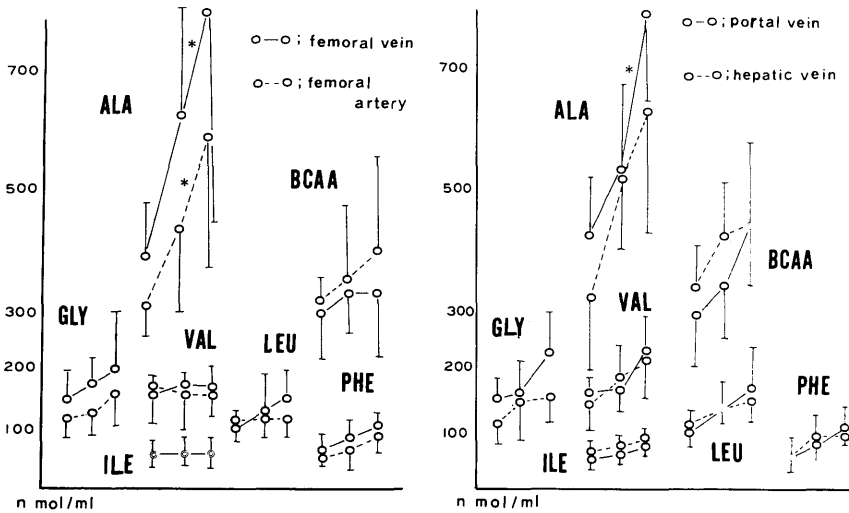


Fig. 8. Free amino acids concentration (EX)
(ALA: alanine GLY: Glycine ILE: isoleucine LEU: leucine
PHE: phenylalanine BCAA: branched chain amino acid)

ed at 3 hours in both groups. PHE increased at 3 hours in EX group despite no significant rise in EX+G one. There was no pronounced variation in BCAA.

Difference in amino-acid level and blood sugar between portal and hepatic veins (Fig. 12): There was a similar tendency for ALA and GLY to be in uptake.

There was significant difference in GLY at 3 hours in EX group and in GLY at 3 hours in EX+G one. BCAA showed a tendency toward release in EX group. The values

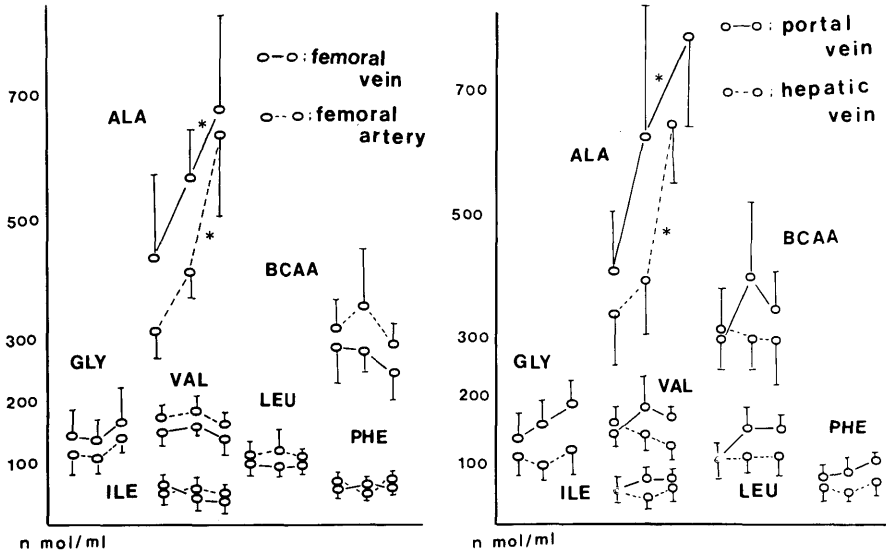


Fig. 9. Free amino acids concentration (EX+G)

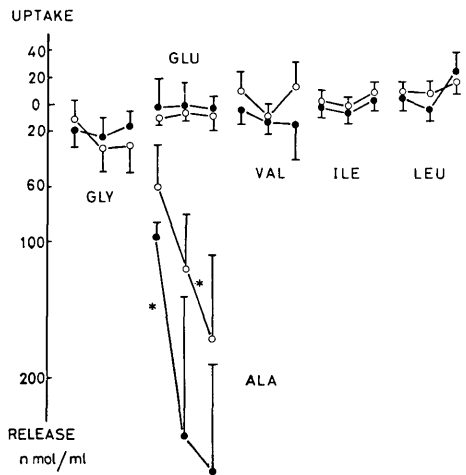


Fig. 10. Femoral-arterio venous differences of amino acids

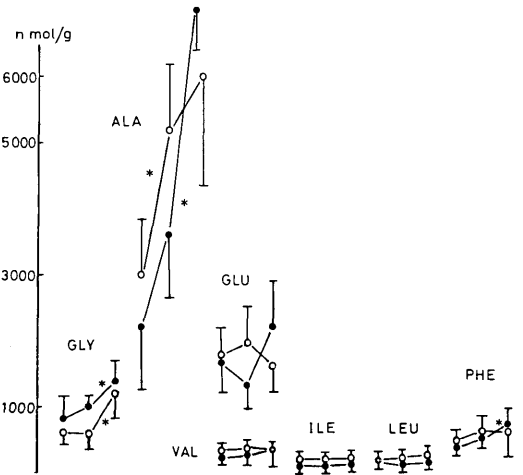


Fig. 11. Amino acid concentration of skeletal muscle

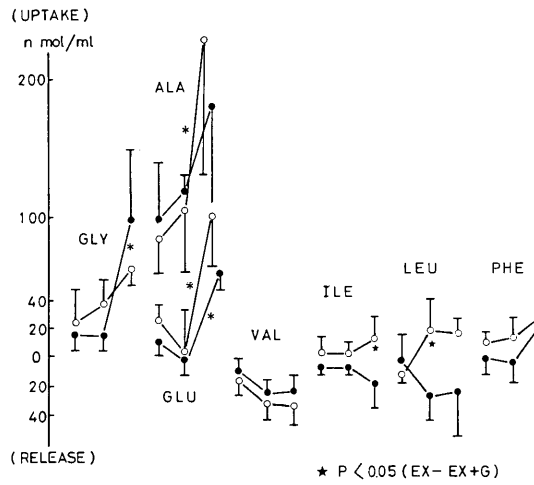


Fig. 12. Porto-hepatic venous differences of amino acids

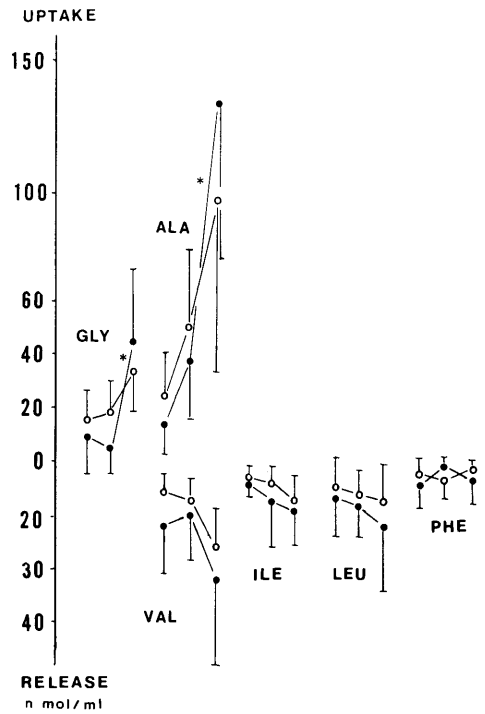


Fig. 13. Arterio-hepatic venous differences of amino acids

of ILE at 3 hours and LEU at 30min were significantly different between both groups. The blood sugar levels reduced, indicating the trend to a release with varying variety in EX+G group.

Difference in amino-acid levels between hepatic artery and vein (Fig. 13): Changes in ALA showed the same trend to be incorporated as those in GLY, although a mode of BCAA variation was almost the releasing tendency. However, there was no statistically significant difference.

Measurement of tissue amino-acid in the liver (fig. 14): The levels of ALA in both groups and LEU and/or PHE in EX group were increased. Generally they were no significant difference.

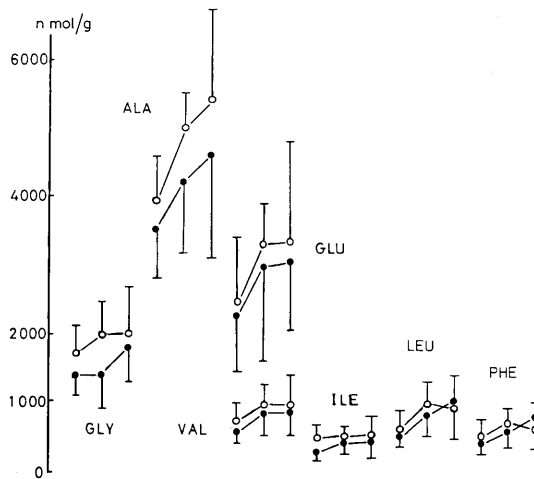


Fig. 14. Amino acids concentration of liver

DISCUSSION

In an attempt to induce an experimental endotoxic shock to animals, the dosage, the administration route of endotoxin or living organism and the host conditions are most influential factors on the severity of ensuing shock. The experimental and clinical shock states would be modified by various factors. Therefore, the means inducing shock are the background for a variety of shock states.

In the present study, induction of shock was made by giving endotoxin (LD100 DIFCO). In view of blood flow, at initiation of shock hypotension resulted in a transient decrease with recovery in the blood flow of the femoral artery and the portal vein. However, recovery from a decrease of blood flow in the portal vein was retarded as compared with that in the femoral artery.

It is suggested that splanchnic pooling might be introduced at the initial stage and

the target organ in shock would be the portal vein in dogs, which is consistent with SILLEHEI' report.¹⁹⁾ The mechanism of a phenomenon of splanchnic pooling is explained by outflow block of hepatic circulation due to constriction of the hepatic vein wall. Viewed from GOT and GPT variations based on acid-base study, the result seemed to indicate that organ functions including the liver and the heart might be impaired with advancing shock state, demonstrating that metabolic acidosis is under way. It is of interest to emphasize that in this study the arterial PCO₂ values did not so vary as usually seen. The reason is that respiratory control by respirator inhibits progressing in respiratory failure, a part of multiple organ failure. It indicates that early care for respiratory failure is necessary to prevent advance in multiple organ failure.

The lactate and pyruvate levels in blood were also raised with elapsing time of shock. It is a reflection that anaerobic metabolism is in progress. It is taken into consideration that endotoxic shock precipitates, anaerobic metabolism much more in tissue than does hemorrhagic one, although SCHLOERB²⁰⁾ has reported that lactate values in endotoxic shock remain high than those in hemorrhagic one under the same magnitude of hypotension. Oddly enough, although excess lactate in EX group significantly increased at 30min, such a trend failed to be observed at 3 hours. Previous investigators have speculated that blood sugar level decreased in proportion to progressing of bacteremia and endotoxic shock caused by gram-negative organism. There are three factors involved in this mechanism; 1) impaired glyconeogenesis,²¹⁾ 2) increasing glucose utilization²²⁾²³⁾ and 3) abnormal insulin secretion. It is believed that glucose is usually utilized in the muscle and nerve tissues, and release to the blood flow as a lactate, followed by synthesizing glucose again (Cori cycle²⁴⁾). A glucose-alanine cycle cited by CAHILL²⁵⁾²⁶⁾ in 1970 is that lactate is converted into alanin via pyruvate to help enzymatic activity of transaminase. It occupies one quarter or one fifth of a total of CORI cycle.²⁷⁾ Generally speaking, it is evident that host metabolism in sepsis is enhanced to strengthen the host's defensive ability with an increase in energy expense, hypersecretion of various hormones, promotion of the process in glycolysis and glyconeogenesis by conversion from lactate and amino-acid. It is clear that the skeletal muscles act as an origin of amino-acid production via the circle of proteolysis with an aid of liver function. Release of AAA and sulfate is undoubtedly indicates the proteolysis on the metabolic course of the peripheral muscles, because no degradation occurs on their courses.²⁸⁾ In this study, changes in PHE were observed as an indicator²⁹⁾ capable for judging amino-acid metabolism in the muscular tissues. Of interest is the fact that utilization of body protein is accelerated in endotoxic shock as a result of increased release of PHE. It is known that BCAA elaborated by degradation of muscle-containing protein is utilized in muscular tissues by oxidation via glucose-alanine cycle. Meanwhile, data from this study indicated that uptake of LEU into the muscular tissues was worthy of notice, although that of VAL and ILE had been negligible. The amino-acid level in

muscular tissues did not change so much. However, difference in the concentration of ILE and LEU between portal and hepatic veins is more likely to imply that ILE and LEU were easy to be incorporated, reflecting that uptake of BCCA differs each other.

SUEGEK *et al.*³²⁾ assume that each amino-acid is metabolized by different metabolic processes, namely, LEU is in the course of acetyl CoA to move into Krebs cycle dependent of NAD, ILE in both acetyl CoA and Succinyl CoA and VAL in succinyl CoA.

The data obtained in this present study indicate that impairment of NAD activity may cause a different modality of up take of BCAA. It has long been known that ALA migrate between organs in body as an end product and plays a Key role in substance of glyconeogenesis.³³⁾³⁴⁾

It is conceivable that ALA takes an important part in detoxication of ammonia,³⁵⁾ inhibition of pyruvate kinase activity³⁶⁾ and promotion of glucagon secretion.³⁷⁾

It is clear that various stress such as starvation,³⁸⁾ serious infection,¹⁶⁾³⁹⁾ major surgery and burn accompany release of ALA from the skeleton muscles, which is easily incorporated into the liver as clearly indicated in this study. This phenomenon, however, was not influenced by hyperosmolar glucose infusion.

Based on a result after comparing the amino-acid levels between portal and hepatic vein, it was noted that uptake of ALA and GLY into the liver was manifest. On the contrary, in view of blood sugar level, it decreased at 3 hours even in EX+G group in spite of showing an increase at 30min. It is a reflection of impairment of glucose metabolism in endotoxic shock. IRI levels well correlated with blood sugar values.

BLACKBURN *et al.*⁴⁰⁾ reported that insulin activity was facilitated by giving glucose, followed by loss of energy production by inhibition of fat metabolism secondary to impairment of endogenous fat utilization in sepsis. In contrast, WICHTERMAN *et al.*¹⁵⁾ identified that glucose utilization in sepsis would much more be precipitated with increase of insulin sensitivity. An explanation can be given from this study that utilization of BCAA and promotion of glyconeogenesis⁴¹⁾ are not benefited from hyperosmolar glucose administration. Much study should be accumulated to solve a problem about substance supplemented for deficit of energy needed in endotoxic shock.⁴²⁾⁴³⁾⁴⁴⁾

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