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Changes of Some Carbohydrates and Amino Acids in the Blood of the Eel after Feeding

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Abstract

Changes of glucose, lactate, pyruvate, and amino acids in the blood of the cultured eel after feeding were investigated. Blood glucose was the highest at 2 hours after feeding. Lactate concentration in the eel blood after feeding increased slowly and pyruvate concentration was the highest at one hour. The ratio of lactate to pyruvate in the eel blood was 35 to 130, which was much higher than that in rat blood. Every amino acids except histidine, tryptophan, glutamate, and aspartate increased remarkably after feeding. Amount of taurine in the eel blood was 100 times higher than that in human blood.

Investigating the changes of some materials in the blood of animal after feeding provides important informations on digestion and absorption, and metabolisms in animal. We investigated the changes of glucose, lactate, pyruvate, and amino acids in the blood of the cultured eel *(Anguilla japonica)* after feeding. It is known that in normal human after feeding blood glucose is the highest after one hour. Although there are some reports on the changes of blood glucose of fishes after feeding[1,2], its change of the eel is still unknown. Chaanges of lactate and pyruvate in the blood are interesting from the viewpoint of gluconeogenesis, since both are good substrates for gluconeogenesis in liver. In the eel liver gluconeogenetic pathway is different from that in mammals described previously[3]. Informations of changes of amino acids in the blood provides useful knowledge on amino acid metabolism.

Protein concentration and transaminase activities in the blood beside materials described above were also investigated.

Experimental Procedures

Eels Cultured eels were obtained from Mr. Ushinohama who cultured eels at Sendai in Kagoshima. Experiments were done every month from May, 1981 to January, 1982. The average weight and length of eels used were shown in Table 1.

Preparation of blood sample Eels were anaesthetized by 2% urethane. The abdomen was cut from the anus to the head by scissors and the hepatic vein between the heart and the liver was cut. After cutting the hepatic vein the blood was collected immediately by syringe containing

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Table 1. Average weight and length of the cultured eels used for experiments. Values were the mean ± s.d. of 30 eels except values of May and Dec. Values of May and Dec were the mean of 25 and 35 eels, respectively.

Month	Weight (g)	Length (cm)
May	187 ± 36	46.8 ± 3.1
Jun	107 ± 20	40.0 ± 1.6
Jul	152 ± 30	43.6 ± 2.3
Sep	151 ± 25	43.6 ± 2.4
Oct	174 ± 13	45.6 ± 2.0
Nov	172 ± 22	45.2 ± 1.8
Dec	172 ± 28	45.1 ± 2.4
Jan	192 ± 31	47.9 ± 2.9

one ml of 0.02% heparin solution. After measuring the volume of the blood the blood was divided into two centrifuge-tubes. One tube contained 0.8 ml of 1 N perchloric acid per ml of blood previously and the other contained nothing. Both tubes were centrifuged at 3,000 rpm for 10 min. The supernatant of the former tube was neutralized with 0.2 ml of 2 M K_2CO_3 per ml of blood. Neutralized supernatant was frozen in liquid nitrogen and used for determination of glucose, lactate, pyruvate, and amino acids. The supernatant of the latter tube was frozen immediately in liquid nitrozen and used for determination of protein and transaminase activities. All samples were stored at freezer of −20°C until samples were analyzed.

Methods of determination Glucose, lactate, and pyruvate were determined by enzymatic methods[4,6]. Amino acids were determined by amino acid analyzer (Hitachi Amino Acid Analyzer Type 835). Protein was determined by the method of biuret[7]. Transaminase activities were measured according to the method of Segal[8] for alanine transaminase and the method of Morino[9] for aspartate transaminase. One enzyme unit is defined as the amount of enzyme catalyzing a decrease of one micromole of NADH per minute under the assay conditions.

Materials Hexokinase, glucose–6–phosphate dehydrogenase, lactate dehydrogenase, and malate dehydrogenase were obtained from Boehringer Mannheim. ATP, NAD^+, $NADP^+$, and NADH were obtained from Oriental Yeast Co. Triethanolamine HCl was purchased from Merck Co. and other reagents were from Wako Pure Chemical Industries.

Results and Discussion

Changes of blood glucose After feeding the blood glucose of the eel increased for 2 hours, then it decreased as shown in Fig. 1. Shimeno reported that the blood glucose of jack mackerel attained the highest from 2 to 3 hours after feeding[1]. The blood glucose of red sea bream after feeding was the highest at 2 hours as well as the blood glucose of the eel[2].

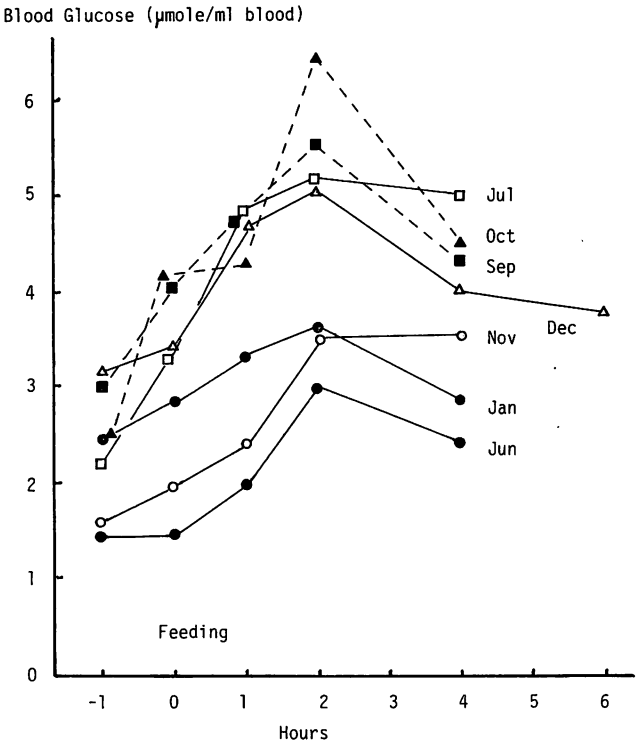

Fig. 1. Chages in concentration of the blood glucose of the eel after feeding. Each value was the mean of 5 eels.

Comparing with the blood glucose of normal mammals, fish blood glucose increased late by one hour after feeding. It is uncertain that in fishes the absorption of glucose after digestion or the utilization of glucose in tissues is slower than that in mammal.

Concentration of the blood glucose of the eel ranged from 1.5 to 6.5 μmoles per ml of blood. These values were lower than those in mammals, jack mackerel or red sea bream.

Changes of lactate and pyruvate Concentration of blood lactate of the eel obtained in summer (June, July, and September) was higher than that in winter (November, December, and January). As shown in Fig. 2 blood lactate of the eel obtained in summer increased after feeding by 2 to 3 times and high concentration of blood lactate continued for 4 hours after feeding. Whereas lactate concentration of the eel obtained in winter did not change remarkably after feeding. The lactate concentration of the eel blood ranged from 1 to 6 μmoles per ml of blood.

The change of pyruvate concentration after feeding was shown in Fig. 3. After feeding pyruvate concentration attained the highest at one hour. After 2 hours the level of pyruvate returned to the level before feeding. The range of the pyruvate concentration of the eel blood was from 0.02 to 0.055 μmole per ml of blood.

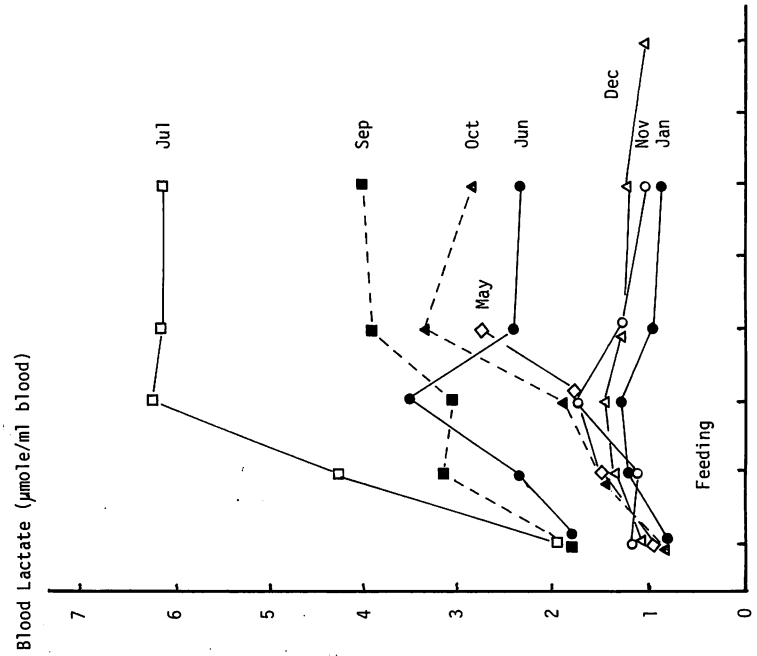

Fig. 2. Changes in concentration of the blood lactate of the eel after feeding.

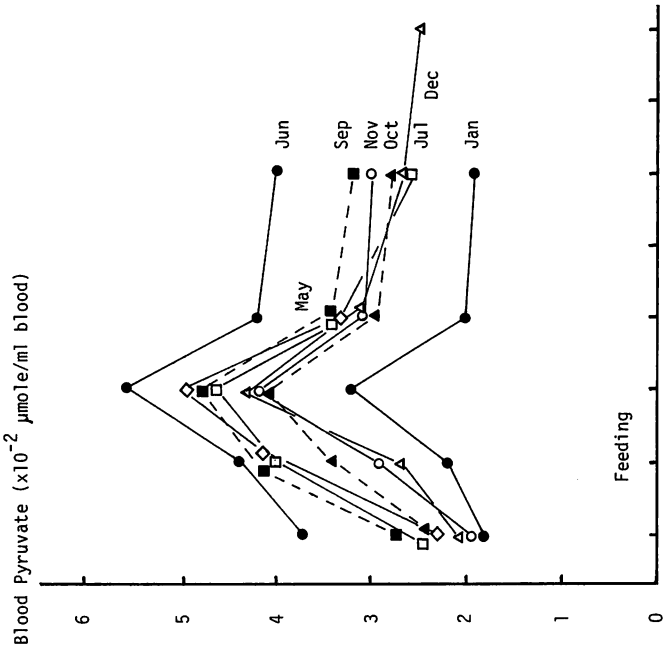

Fig. 3. Changes in concentration of the blood pyruvate of the eel after feeding.

The ratio of lactate to pyruvate in the blood of the eel was from 35 to 130. These values were very high comparing with those in rat blood. In rat blood the ratio was from 11 to 12[10]. The high ratio is due to rather high level of lactate and low level of pyruvate in the eel blood. Recently we have clarified that the type of lactate dehydrogenase in the eel liver is heart type. Whereas it is known that lactate dehydrogenase in rat liver is muscle type[11]. Heart type lactate dehydrogenase unlike muscle type one proceeds mainly the conversion of lactate to pyruvate. Pyruvate in liver cells is utilized for gluconeogensis or for energy through TCA-cycle. It is interesting whether the high ratio of lactate to pyruvate in the eel blood implicates or does not in gluconeogenesis and energy metabolism in the eel liver.

Changes of amino acids Changes of the concentration of each amino acids in the blood of the eel obtained in June was investigated. Every amino acids except histidine, tryptophan, aspartate, and glutamate increased remarkably after feeding as shown in Fig. 4. Methionine, leucine, isoleucine, and alanine increased by 18, 13, 12, and 9 times, respectively at 4 hours after feeding. Total amino acids increased 4 times at 4 hours after feeding.

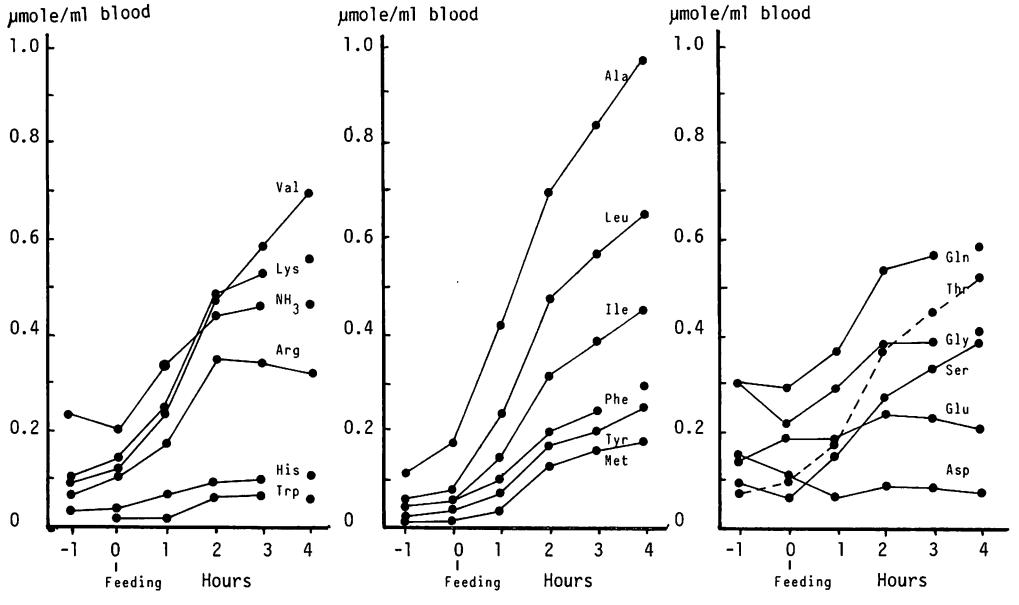

Fig. 4. Changes in concentration of the amino acids of the eel after feeding.

Low amount of histidine, tryptophan, aspartate, and glutamate seemed due to their permeability to the cell membrane. The amount of tryptophan and histidine seems to be important from the viewpoint of the nutrition of the eel. However, since the blood used for analysis was collected from the hepatic vein as described in "Experimental Procedures", considerable amount of amino acids seems to be utilized in the liver. It is known that the

difference of the amonunt of alanine between portal and hepatic vein of rat is 149 μM[12].

Among amino acids of the eel blood taurine was 5.55 μmoles per ml of blood as shown in Table 2. This value was about 100 times higher than the amount of taurine in human blood. It is unknown why such a high concentration of taurine is contained.

Table 2. Cmparision of the amount of each amino acids in the eel and human blood. Eels before feeding by one hour were used.

Amino Acid	Eel* μmole/ml blood	Human** μmole/ml blood
Tau	5.55 ± 1.35	0.05
Asp	0.15 ± 0.09	0.15
Thr	0.08 ± 0.03	0.16
Ser	0.10 ± 0.03	0.12
Glu	0.14 ± 0.05	0.03
Gln	0.30 ± 0.27	0.63
Gly	0.30 ± 0.13	0.25
Ala	0.11 ± 0.04	0.39
Met	0.01 ± 0.01	0.04
Ile	0.03 ± 0.01	0.07
Leu	0.06 ± 0.01	0.14
Tyr	0.03 ± 0.01	0.08
Phe	0.04 ± 0.01	0.06
Lys	0.10 ± 0.04	0.17
His	0.03 ± 0.01	0.09
Arg	0.07 ± 0.00	0.10
Val	0.09 ± 0.02	0.24

* Values of eels were the mens ± s.d. of 5 eels.

** Biochemical Data Book (1979) 1548 (Tokyo Kagaku Dojin, Tokyo).

Protein and transaminase in the blood No change of protein concentration and transaminase activities in the plasma of the eel was observed after feeding as shown in Fig. 5 and Fig. 6. However, seasonal variation was observed in protein concentration and transaminase activities. Especially alanine transaminase activities in September and July were high and those in May and January were low.

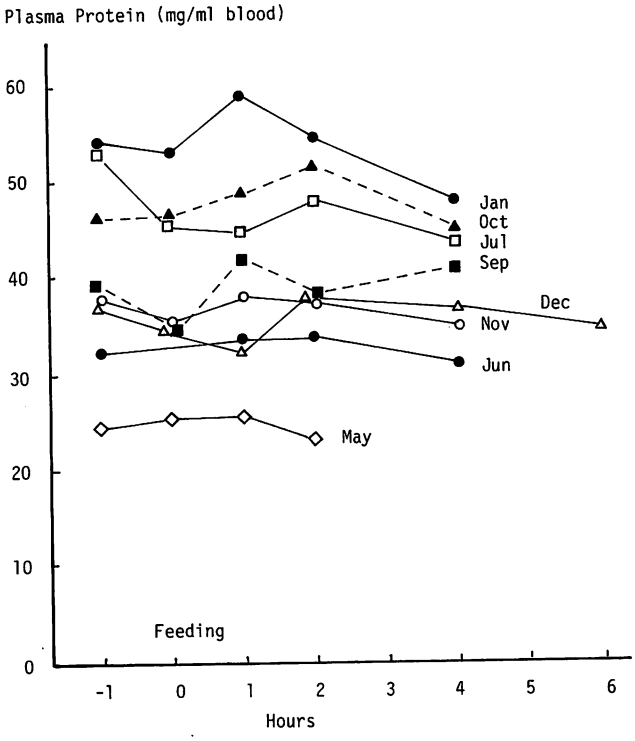

Fig. 4. Changes in concentration of the plasma protein of the eel after feeding.

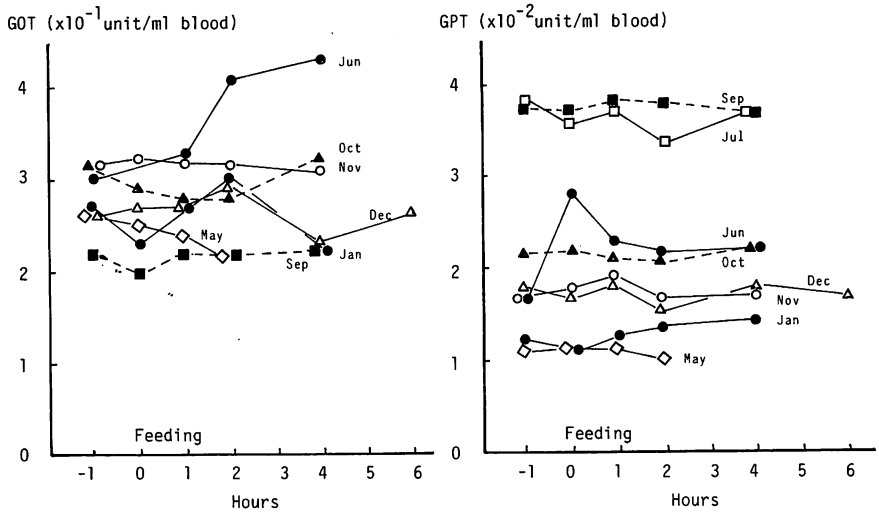

Fig. 6. Changes of transaminase activities in the eel blood after feeding.

Acknowledgment

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