Incorporation of ^{14}C -Lactate into Glucose by Perfused Eel Liver

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The incorporation of ^{14}C -lactate into glucose by the isolated perfused eel liver was investigated. The rate of synthesis of ^{14}C -glucose was steady for 120 min and the mean time for conversion of lactate to glucose was about 60 sec. The rate of incorporation of ^{14}C -lactate into glucose was 5.5% in the case of a recirculating system and 14.1% in the case of a nonrecirculating system.

Many teleost species are known to tolerate for a long period of starvation, and gluconeogenesis in their livers is stimulated during starvation.^{1,2)} In a previous investigation using isolated perfused liver of the eel *Anguilla japonica*, we have shown that the rate of glucose synthesis from excess lactate is almost the same as that in mammals.³⁾ However, colorimetric determination of glucose has been unsatisfactory to investigate the effect of glucagon, epinephrine or 3', 5'-c-AMP on gluconeogenesis, since conversion of glycogen into glucose was also stimulated in starved eel livers by these drugs.

This report describes the method to study gluconeogenesis by the isolated perfused liver of the eel using ^{14}C -lactate.

Experimental Procedure

Perfusion of the isolated liver Eels (90 to 120 g) were obtained from fish market. They were kept in a brackish water tank and fasted for 3 to 4 months before use.

The perfusion apparatus and procedure have been described previously.³⁾ The perfusion medium, KREBS-RINGER bicarbonate buffer (pH 7.4 at 25°C), was freshly made each day and oxygenated continuously with O_2 and CO_2 (95:5). Albumin and hemoglobin or erythrocytes were not included in the medium. In all experiments livers were initially perfused for 30 minutes with a recirculating medium. At the end of this period, sodium DL-lactate (20 mM, Wako Chemicals) and trace amount of sodium DL-2- ^{14}C -lactate (29 mCi/mmol, Amersham-Searle) were added to the medium in the reservoir (50 or 100 ml). In some experiments, in order to maintain a constant substrate level the perfusion was changed to a nonrecirculating or "flow-through" system and 2 mM of lactate was added.

Separation of glucose and lactate in the perfusate The separation of ^{14}C -glucose synthesized from ^{14}C -lactate was as follows. Aliquots (1.0 ml) of perfusate was applied to

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double columns, the one column being set upon the other, and each column (inner diameter 0.65 cm) was consisted of two layers of resins (lower layer, Dowex 50 $\times$ 8, H⁺ form, 200 to 400 mesh, 1 cm; upper layer, Dowex 1 $\times$ 4, HCOO⁻ form, 100 to 200 mesh, 2 cm). 0.5 ml of 10 mM glucose was applied to columns as carrier and the first 1.5 ml of eluate was discarded. Then the columns were washed with 2.5 ml of water. The washing solution was collected. By this method ¹⁴C-lactate present initially in the medium was removed more than 99%. In the case of preparation of samples for paper chromatography of ¹⁴C-glucose, the size of column, which was also consisted two layers of resins, was 1.8 $\times$ 14.5 cm.

Determination of radioactivity 1.0 ml of the washing (2.5 ml) was added to 10 ml of toluene containing 4 g of 2, 5-diphenyloxazole (PPO), 0.1 g of 1, 4-bis [2-(5-phenyloxazolyl)] benzene (POPOP) and 500 ml of Triton X-100 per liter and the radioactivity was determined in a liquid scintillation spectrometer (Beckman LS-230).

The detection of radioactivity on paper chromatogram was carried out by counting the radioactivity of papers cut into pieces, which were added to 5 ml of scintillation fluid (4 g of PPO and 0.1 g of POPOP per liter of toluene).

Paper chromatography The fraction unadsorbed by ionexchange resins was identified as ¹⁴C-glucose by paper chromatography using the solvent system; *n*-butanol: pyridine: water = 6: 4: 3. The development was carried out by descending technique at room temperature. After development, papers were sprayed to detect glucose with the solvent; 1.7 g phthalate and 0.93 g aniline in 100 ml of *n*-butanol saturated with water. To detect

Fig. 1. Paper chromatogram of the fraction unadsorbed by resins (Dowex 1 and Dowex 50). Solvent system was *n*-butanol: pyridine: water = 6: 4: 3. Glucose was added as carrier.

lactate papers were sprayed with 0.05% bromophenol blue. After spraying papers were dried at 105°C for 5 to 7 min. Glucose appeared as brown and lactate as blue.

Results

Identification of ^{14}C -glucose synthesized from ^{14}C -lactate A liver (0.7 g) was perfused for 120 min with 20 mM sodium DL-lactate and sodium DL-2- ^{14}C -lactate (0.85 μCi). At the end of this period 10 ml of perfusate and 50 μmoles of glucose as carrier were applied to the column. 0.1 ml of the unadsorbed fraction, which was evaporated with rotary evaporator and dissolved in 1.0 ml of water, was applied to the paper. In the present system, R_f values of glucose and lactate were 0.45 and 0.20 respectively. As shown in

Fig. 2. Time course of ^{14}C -glucose production from DL-2- ^{14}C -lactate. Livers (0.7–1.0 g) from fasted eels were perfused for 120 min with recirculating medium containing 20 mM DL-lactate and DL-2- ^{14}C -lactate (0.85 μCi). The means of three observations are shown with 2 times of the standard errors of the mean (vertical bars).

Fig. 1, radioactivity of the paper chromatogram was corresponded to glucose.

Time course of gluconeogenesis from ^{14}C -lactate Livers (0.7 to 1.0 g) were perfused with 20 mM DL-lactate and DL-2- ^{14}C -lactate (0.85 μCi). As shown in Fig. 2 the time course of the incorporation of ^{14}C -lactate into glucose was steady for 120 min.

Time for conversion of lactate to glucose After a liver was perfused with 10 mM DL-lactate for 10 min, a pulse of DL-2- ^{14}C -lactate (0.43 μCi in 50 μl) was injected into the medium flowing into the liver, and the outflowing perfusate was collected at intervals. The mean time for conversion of lactate to glucose was calculated from the difference between the peaks of total radioactivity and of radioactivity in glucose, and was 60 ± 15 sec (Fig. 3).

Fig. 3. Time for conversion of lactate to glucose. A liver of a fasted eel was perfused for 10 min with medium containing 10 mM sodium DL-lactate in a recirculating system. Sodium DL-2- ^{14}C -lactate (0.43 μCi in 50 μl) was then rapidly injected into the medium flowing into the liver, and samples of the effluent were collected at intervals.

Incorporation of ^{14}C -lactate into glucose by a nonrecirculating system The concentration of substrates inevitably changes during perfusion in a recirculating system, while in a nonrecirculating system it could be maintained steady, since the perfusion fluid passes through the liver only once. The incorporation of ^{14}C -lactate into glucose by a recirculating system and by a nonrecirculating system was 5.5% and 14.1% respectively (Table 1).

Table 1. The rate of ^{14}C -glucose formation in perfused eel livers by recirculating and nonrecirculating system

System	^{14}C -Glucose (%)
Recirculating ¹⁾	5.47 $\pm$ 0.31
Nonrecirculating ²⁾	14.13 $\pm$ 0.40

1) DL-lactate 22 mM DL-2- ^{14}C -lactate 0.85 μCi

2) DL-lactate 2 mM, DL-2- ^{14}C -lactate 0.85 μCi

$$\% = \frac{^{14}\text{C-glucose dpm/g liver/hr}}{\text{total dpm of } ^{14}\text{C-lactate added initially}} \times 100$$

Discussion

^{14}C -glucose in the perfusate was separated from lactate by small columns of Dowex 1 $\times$ 4 (HCOO^-) and Dowex 50 $\times$ 8 (H^+), more than 99% of ^{14}C -lactate present initially in the medium being removed. The radioactivity of the fraction unadsorbed by resins was identified as glucose by paper chromatography. Although we tried the method of EXTON *et al.*⁴⁾ to remove ^{14}C -lactate and other charged compounds in the medium, it was found that the recovery of glucose and the removal of ^{14}C -lactate were unsatisfactory. This seemed partially owing that we used Dowex 1 $\times$ 4 (OH^- form, 100 to 200 mesh) instead of Duolite A-4 (OH^- form, 10 to 50 mesh).

As shown in the present study, the mean time for conversion of lactate to glucose in the perfused eel liver was about 60 sec. EXTON *et al.*⁴⁾ reported that the time for conversion of lactate to glucose in the perfused rat liver about 75 sec. The rate of incorporation of ^{14}C -lactate into glucose per g eel liver per hour was 5.5% in the case of a recirculating system and 14.1% in the case of a nonrecirculating system. These results indicate that the synthetic function of glucose in the perfused eel liver is almost same as in mammals.⁴⁾

The eel used for the present experiments was fasted for 3 to 4 months. In spite of these long periods of starvation eels were able to survive and to accelerate gluconeogenesis in liver. It will be interesting to investigate metabolic regulation of gluconeogenesis in the liver of eel during starvation.

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