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Non-invasive sampling of lactic acid ions by iontophoresis using chloride ion in the body as an internal standard

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Abstract: Non-invasive sampling of lactic acid, as a model endogenous compound, through hairless rat skin by iontophoresis was investigated using a two-chamber iontophoretic diffusion cell equipped with platinum electrodes and a pulse depolarization iontophoretic system. Chloride ion in the body was used as an internal standard. First, an *in vitro* experiment on the permeation of lactate and chloride ions through hairless rat skin was carried out to determine the flux ratio of these ions. The cathode side of the cell (dermis side) was filled with physiological saline containing lactic acid (0.5556, 1.111, 1.667 or 2.222 mmol cm^{-3}) and the anode side (epidermis side) with phosphate buffer (pH 7.4). The amount of lactate and chloride ion permeated from the dermis side to the epidermis side through the skin at a constant current of 3.0 mA was determined using an automatic lactic acid analyser and high-performance ion chromatography, respectively. For construction of a calibration curve of lactic acid in the dermis side, the ionic mobility ratio of lactic acid/chloride ion (U_{Cl}/U_{lac}) was determined using a computer simulation program from the flux ratio of lactic acid and chloride ion and the applied concentration of lactic acid in the dermis side. Second, an *in vitro* non-invasive sampling experiment of lactic acid through rat skin was carried out at a constant current of 2.0 or 3.0 mA and 2.222 or 1.111 mmol cm^{-3} of lactic acid in the dermis side, respectively. The lactic acid concentration calculated from the flux ratio of lactate and chloride in this experiment reflected the applied concentration of lactic acid in the dermis side in spite of different applied currents. Non-invasive sampling of endogenous ion by iontophoresis using chloride ion as an internal standard reflected the concentration of endogenous ion in spite of changes in applied current and/or skin resistance.

Keywords: *Iontophoresis; lactic acid; chloride ion; hairless rat skin; non-invasive sampling; chloride ion as internal standard.*

Introduction

The detection and/or quantification of endogenous chemicals is a key factor in any medical diagnosis. Quantification of the blood level of xenobiotics, often known as therapeutic drug monitoring, is important in determining a suitable dosage regimen for an individual patient and preventing side-effects. These tests of endogenous and exogenous chemicals are usually made by blood sampling with a needle, a method which is invasive and requires another individual to take the sample. Pain may accompany the sampling procedure, and there may be a risk of bacterial or viral infection for both patient and sampler. In diabetic patients, a self controlled means is available by which the blood glucose level can be quantified and insulin administered by the patients themselves. All that is needed is a small punch for blood sampling, a reagent-coated test paper strip and a blood glucose measuring apparatus. The patient first pierces a finger to get a drop of blood, which is then put on the test paper. Glucose in the blood reacts with the reagent in the paper. The paper strip is then inserted into the blood glucose measuring apparatus to obtain the blood glucose level. The method is simple but nonetheless may be difficult for a child or an aged patient to use.

Iontophoresis, a potential non-invasive technique of drug administration, can be defined as a process of moving a charged or ionized drug across a biological membrane using an electric current [1]. It has gained much attention because it is one of the few non-invasive methods by which a drug can be administered through a biological membrane. For iontophoretic transdermal drug delivery, an electric current is generally applied to enhance the movement of a charged or ionized drug from the epidermis to the dermis. Movement of a neutral compound was reported

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recently to have been enhanced by this method [2]. An endogenous ion and a drug can, in a reverse process, be collected from the body through the skin. Glikfeld *et al.* reported the application of iontophoresis for the non-invasive sampling of glucose with high flux from the dermis to the epidermis [3]. Unfortunately, their results suggested that endogenous materials collected by this method may not always reflect precisely their concentrations in the body. One possible reason is skin damage that may occur during application of the current. Skin barrier function was changed as a result of damage to the skin with a higher current [4], and the current used may itself change during the application. It is therefore, difficult to determine the concentration of endogenous materials in the body when the flux of these materials is altered either by skin resistance and/or the current applied during iontophoresis.

In the present study, the non-invasive sampling method has been improved and an attempt has been made to determine the concentration of lactic acid in the dermis side of the cell by employing the flux ratio of lactic acid and chloride ion (a constant level endogenous ion). The aim of the study was to improve the iontophoretic sampling method even where changes occur in applied current and/or skin resistance.

Theory

The total electric current, I_{tot} (mA) through the membrane obeys the following relationship [5]:

$$I_{tot} = Z_{lac}\cdot F\cdot J_{lac}\cdot S + Z_{Cl}\cdot F\cdot J_{Cl}\cdot S + Z_c\cdot F\cdot J_c\cdot S, \quad (1)$$

where Z, F and S are the charge of the ion, Faraday constant ($9{\cdot}6485 \times 10^4$ C mol^{-1}) and surface area of the membrane available for ion permeation (cm^2), respectively, and J_{lac}, J_{Cl} and J_c are the flux of lactic acid, chloride ion and total counter ion (mmol cm^{-2} s^{-1}), respectively. The flux of ion i, J_i, through the membrane can be obtained using the following equation:

$$J_i = C_i\cdot U_i\cdot E, \quad (2)$$

where C_i, U_i and E are the concentration (mmol cm^{-3}) of the ion i, ionic mobility (cm^2 s^{-1} V^{-1}) of the ion i and potential gradient (V cm^{-1}), respectively. Using equation (2), equation (1) can be transformed to:

$$I/S = Z_{lac}\cdot F\cdot C_{lac}\cdot U_{lac}\cdot E + Z_{Cl}\cdot F\cdot C_{Cl}\cdot U_{Cl}\cdot E + Z_c\cdot F\cdot C_c\cdot U_c\cdot E. \quad (3)$$

When a constant concentration of counter ion (C_c) in the epidermis side is applied, the sum of lactic acid and chloride ion flux ($J_{lac} + J_{Cl}$) should be constant, as previously reported [6]. The sum of the transport number of lactate and chloride ion is obtained by:

$$t_{(lac+Cl)} = (Z_{lac}\cdot F\cdot J_{lac} + Z_{Cl}\cdot F\cdot J_{Cl})/(Z_{lac}\cdot F\cdot J_{lac} + Z_{Cl}\cdot F\cdot J_{Cl} + Z_c\cdot F\cdot J_c), \quad (4)$$

where $t_{(lac+Cl)}$ is the sum of the transport number of lactate and chloride ion. When the total constant current applied is changed, $t_{(lac+Cl)}$ remains constant, which means that the ratio of anion flux contributing to the total current is also constant and is independent of the total constant current. The flux ratio of lactate and chloride ion (R_{lac}) from the dermis to epidermis side is defined as:

$$R_{lac} = Z_{lac}\cdot F\cdot J_{lac}/(Z_{lac}\cdot F\cdot J_{lac} + Z_{Cl}\cdot F\cdot J_{Cl}). \quad (5)$$

Using equation (2), equation (5) is transformed to:

$$R_{lac} = Z_{lac}\cdot F\cdot C_{lac}\cdot U_{lac}\cdot E/(Z_{lac}\cdot F\cdot C_{lac}\cdot U_{lac}\cdot E + Z_{Cl}\cdot F\cdot C_{Cl}\cdot U_{Cl}\cdot E). \quad (6)$$

Equation (6) shows that the flux of lactic acid (J_{lac}) and chloride ion (J_{Cl}) increases and decreases, respectively when the concentration of lactic acid (C_{lac}) is increased. This can be explained as follows: when C_{lac} increases, the potential gradient (E) across the membrane decreases [equation (6)], because J_i ($J_{lac} + J_{Cl}$) is constant at a constant current and U_i (U_{lac} and U_{Cl}) is the intrinsic coefficient under a constant C_i (C_{Cl}) [equation (2)]. Equation (6) then becomes:

$$R_{lac} = Z_{lac}\cdot F\cdot C_{lac}\cdot U_{lac}\cdot E/(Z_{lac}\cdot F\cdot C_{lac}\cdot U_{lac}\cdot E + Z_{Cl}\cdot F\cdot C_{Cl}\cdot U_{Cl}\cdot E) = C_{lac}\cdot U_{lac}/(C_{lac}\cdot U_{lac} + C_{Cl}\cdot U_{Cl}), \quad (7)$$

where Z_{lac}, Z_{Cl}, F and E are the charge of the lactate and chloride ion (both -1), Faraday constant and potential gradient, respectively. The potential gradient, E can be omitted because E is the same value for both ions. The

lactic acid concentration in the dermis side can be determined using the flux ratio of lactic acid and chloride ion. The flux ratio is constant and is independent of the potential gradient. The concentration of lactic acid (mmol cm^{-3}) using this method can be defined as:

$$C_{lac} = (U_{Cl}/U_{lac}) \cdot C_{Cl} \cdot (R_{lac}/(1 - R_{lac})), \quad (8)$$

where U_{Cl}, U_{lac}, C_{Cl} and R_{lac} are, respectively, the ionic mobility of chloride ion ($cm^2\ s^{-1}\ V^{-1}$), ionic mobility of lactic acid ($cm^2\ s^{-1}\ V^{-1}$), chloride ion concentration (mmol cm^{-3}) and flux ratio of lactic acid and chloride ion. The ionic mobility ratio of chloride ion and lactic acid (U_{Cl}/U_{lac}) is determined from the flux ratio of the two compounds (R_{lac}) and the applied concentration of lactic acid (C_{lac}) using a computer simulation program, MULTI [7]. Equation (8) can be applied when the ionic mobility ratio (U_{Cl}/U_{lac}) is constant. When the skin is damaged, the U_{Cl} and U_{lac} may be changed. If the U_{Cl} changes in parallel with U_{lac}, equation (8) can be used for calibration. That is to say, the ionic mobility of chloride ion (U_{Cl}) through damaged skin increases in contrast to intact skin at the same time as the ionic mobility of lactate ion (U_{lac}). The relationship between the ionic mobilities of chloride and lactate ion (U_{Cl}, U_{lac}) is described in detail below.

Experimental

Materials

Lactic acid (reagent grade) was obtained from Hayashi Pure Chemical Industries (Osaka, Japan). Sodium chloride (reagent grade) was obtained from Wako Pure Chemical Industries (Osaka, Japan). Platinum wire for electrodes (99.9%, purity, 10 mm × 1 mm) was from Tokuriki (Tokyo, Japan). Other chemicals were of reagent grade. All materials were used without further purification. All solutions were made with deionized water which had been passed through a water purifier (Eyla ER, Tokyo Rikakikai, Tokyo, Japan). The resulting water had a pH of 6.74 and an electrical conductivity of 0.39 μs cm^{-1}.

Skin membrane preparation

Excised full-thickness skin of the male hairless rat (WBN/ILA-Mt, 6 weeks old, Life Science Research Center, Josai University, Saitama, Japan) was used.

Power source for non-invasive sampling study by iontophoresis

A CV/CC pulse generator (Matsuzaki Koki, Tokyo, Japan) was used for this non-invasive sampling study. This pulsed depolarization iontophoretic system generates constant pulsed direct current (frequency, 40 kHz; on–off duty, 30%) [8].

Determination of permeated ion

Lactic acid was assayed by an automatic analyser (TDX automated fluorescein polarization analyser, Abbott Laboratories, Irving, TX, USA). A high-performance ion chromatography system comprising a pump (LC-9A, Shimadzu, Kyoto, Japan), a conductivity detector (CDD-6A, Shimadzu) and an integrator (C-R5A, Shimadzu) was used to analyse the chloride ion. The mobile phase consisted of 2.5 mM phthalic acid and 2.4 mM tris-(hydroxymethyl)-aminomethane and the flow rate was 1.5 ml min^{-1}. Chloride ion was resolved using a packed anion exchange column (Shim-pack IC-A1, 100 × 4.6 mm i.d., Shimadzu) with a packed guard column (Shim-pack IC-GA1, 10 × 4.6 mm i.d., Shimadzu) and was determined by measurement of electric conductivity. Sodium fluoride solution (100 μg ml^{-1}) was used as an internal standard.

Calibration

Samples of full-thickness skin, 2 × 2 cm, were mounted between the cathode and anode sides of a two-chamber iontophoretic diffusion cell equipped with platinum electrodes. The cathode (dermis) side of the cell was filled with 4 cm^3 of physiological saline containing lactic acid (0.5556, 1.111, 1.667 of 2.222 mmol cm^{-3}) as a model drug of endogenous ion, and the anode (epidermis) side with 4 cm^3 of phosphate buffer (pH 7.4). The cell set was stirred by star-head bars in both sides of the cell and activated by a constant-speed synchronous motor (MC-301, Scinics, Tokyo, Japan) at 1200 rpm. The electrodes were connected to the power source as shown in Fig. 1. The surface area of the membrane available for the sampling study was 0.95 cm^2; experiments were conducted at 37°C. At a predetermined time, 0.2 cm^3 of anode side solution was removed from the cell and the same volume of

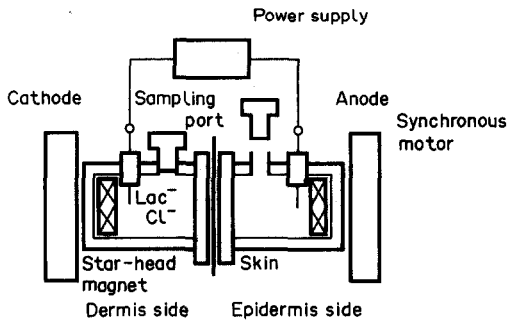

Figure 1
Schematic diagram of lactic acid collection study. In this and other figures Lac^- denotes lactic acid.

phosphate buffer (pH 7.4) was added to maintain the volume in the cell throughout the experiment. The flux of lactate and chloride ion from the dermis side to the epidermis side through the hairless rat skin was determined for each lactic acid concentration at a constant current of 3.0 mA. The flux of each compound was calculated using a steady-state slope in each permeation study (Figs 2 and 3). To construct a calibration curve of lactic acid in the dermis side, the ionic mobility ratio of both substances (U_{Cl}/U_{lac}) was determined using the computer simulation program MULTI from the flux ratio of lactate and chloride ion and the applied lactic acid concentration in the dermis side.

In vitro *non-invasive sampling study of lactic acid by iontophoresis*

Non-invasive sampling of lactic acid through the rat skin was carried out at a constant current of 2.0 or 3.0 mA at a concentration of lactic acid of 2.222 or 1.111 mmol cm^{-3}, respectively, and the flux ratios of lactate and chloride ion were determined. The lactic acid concentration in the dermis side was estimated from these ratios from a calibration curve. The difference between the lactic acid concentration estimated from this newly developed non-invasive sampling method and the applied lactic acid concentration was evaluated.

Results

Calibration of lactic acid by iontophoresis

Figure 2 shows the effect of lactic acid concentration in the cathode side on the permeation of lactic acid from the dermis side to the epidermis side through rat skin at a constant current of 3.0 mA; Fig. 3 shows the

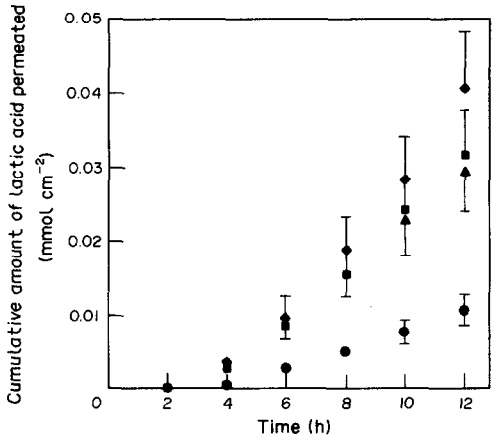

Figure 2
Effect of lactic acid concentration on its permeation through hairless rat skin. ●, 0.5556 mmol cm^{-3}; ▲, 1.111 mmol cm^{-3}; ■, 1.667 mmol cm^{-3}; ◆, 2.222 mmol cm^{-3}. Each point represents the mean ±SE of five experiments.

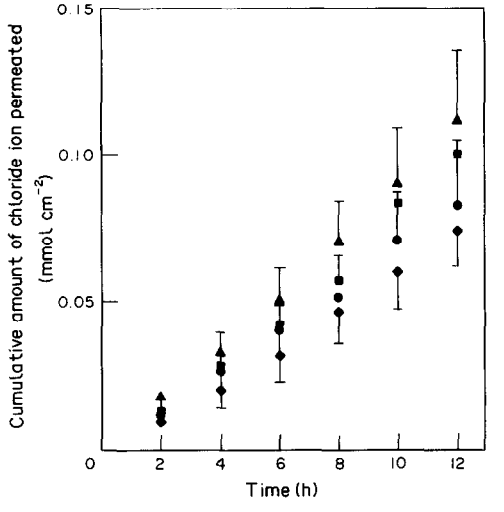

Figure 3
Effect of lactic acid concentration on the permeation of chloride ion through hairless rat skin. ●, 05556 mmol cm^{-3}; ▲, 1.111 mmol cm^{-3}; ■, 1.667 mmol cm^{-3}; ◆, 2.222 mmol cm^{-3}. Each point represents the mean ±SE of five experiments.

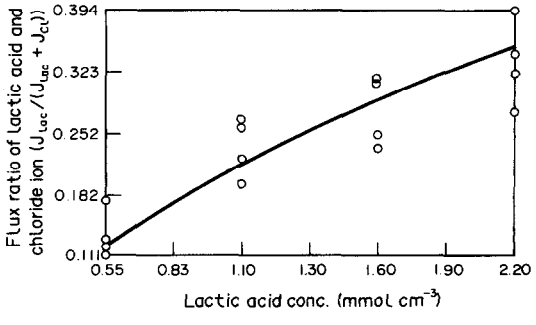

Figure 4
Calibration curve of lactic acid in the dermis side; the solid line is the computer simulation curve.

effect on the permeation of chloride ion under the same conditions. Figure 4 shows the relationship between the fraction of the flux of lactic acid over the total flux of lactic and chloride ion ($J_{lac}/(J_{lac} + J_{Cl})$) and the concentration of lactic acid applied in the dermis side. A solid line obtained by computer simulation was used as to calibrate lactic acid in the dermis side. The ionic mobility ratio of lactate/chloride (U_{Cl}/U_{lac}) calculated by computer simulation was 26.16. Using equation (8), these ionic mobilities and physiological saline concentration (0.1538 mmol cm^{-3}), a calibration curve of lactic acid concentration in the dermis side were defined as:

$$\begin{aligned} C_{lac} &= (U_{Cl}/U_{lac})\, C_{Cl}\, (R_{lac}/(1 - R_{lac})) \\ &= 26.16 \times 0.1538\, (R_{lac}/(1 - R_{lac})) \\ &= 4.023\, (R_{lac}/(1 - R_{lac})), \end{aligned} \quad (9)$$

where C_{lac} (mmol cm^{-3}) and R_{lac} were the concentration of lactic acid in the dermis side and the fraction of the flux of lactic acid over the total flux of lactate and chloride ion ($J_{lac}/(J_{lac} + J_{Cl})$) in the epidermis side, respectively.

In vitro *non-invasive sampling study of lactic acid by iontophoresis*

Table 1 shows the flux of lactate and chloride ion, the fraction of the flux of lactic acid over the total flux of the two materials and the estimated value of the lactic acid concentration in the dermis side using this method with an applied lactic acid concentration of 2.222 mmol cm^{-3} at a constant current of 2.0 mA. Table 2 shows the same data using the same method but with an applied lactic acid concentration of 1.111 mmol cm^{-3} at a constant current of 3.0 mA.

Discussion

When the applied concentration of lactic acid in the dermis side was increased, the flux of lactic acid and chloride ion increased and decreased, respectively, at the constant current of 3.0 mA as shown in Figs 2 and 3. Chloride ion flux decreased with an increase in lactic acid concentration except for a concentration of 0.5556 mmol cm^{-3} of the acid in the dermis side (Fig. 3). This phenomenon is still not well understood, but the skin membrane resistance may be lower than that of the normal state. When the skin membrane resistance decreases, the potential gradient across the skin membrane also decreases at a constant current; the potential gradient is the driving force of the ion. The flux ratio of lactic acid/chloride ion (R_{lac}) was constant and was independent of the potential gradient (E), as shown by equation (7). For this reason the non-invasive sampling technique with chloride ion as an internal standard can be used to estimate the lactic acid concentration whenever the skin is damaged or the applied current is changed. Table 1 shows the estimated value of lactic acid concentration

Table 1
Flux of lactic acid and chloride ion, flux ratio of lactic acid and chloride ion and estimated value of lactic acid for each of five experiments

Experiment* no.	J_{lac} (mmol cm^{-2} h^{-1})	J_{Cl} (mmol cm^{-2} h^{-1})	R_{lac}	C_{lac} (mmol cm^{-3})
1	0.001737	0.003180	0.3532	2.198
2	0.003146	0.004974	0.3875	2.546
3	0.001359	0.001516	0.4728	3.609
4	0.002033	0.002938	0.4890	2.784
5	0.0003663	0.0004588	0.4439	3.211

*Applied lactic acid concentration and constant current were 2.222 mmol cm^{-3} and 2.0 mA, respectively.

Table 2
Flux of lactic acid and chloride ion, flux ratio of lactic acid and chloride ion and estimated value of lactic acid for each of five experiments

Experiment* no.	J_{lac} (mmol cm^{-2} h^{-1})	J_{Cl} (mmol cm^{-2} h^{-1})	R_{lac}	C_{lac} (mmol cm^{-3})
1	0.001330	0.004323	0.2353	1.238
2	0.002172	0.01090	0.1662	0.8019
3	0.002125	0.008402	0.2019	1.018
4	0.001309	0.004736	0.2165	1.112
5	0.001173	0.003583	0.2466	1.317

*Applied lactic acid concentration and constant current were 1.111 mmol cm^{-3} and 3.0 mA, respectively.

in the dermis side using this method when 2.222 mmol cm^{-3} lactic acid was applied in the dermis side at a constant current of 2.0 mA; Table 2 shows the same data set at 1.111 mmol cm^{-3} lactic acid and a constant current of 3.0 mA. Each non-invasive sampling revealed almost the same lactic acid concentration in the dermis side in spite of different applied currents. The relative standard deviation of the results in Tables 1 and 2 was 19.30 and 18.33%, respectively. The cause of these large fluctuations is not clear, but the flux of lactate acid and chloride ion obtained may not represent a steady state and/or co-existing ions may influence the flux values.

In non-invasive sampling of an endogenous ion by constant voltage iontophoresis, the ion flux obeys equation (2). In this equation the flux of ion i (J_i) is proportional to the applied concentration of ion i (C_i) when the ionic mobility (U_i) and potential gradient (E) are constant. The concentration of endogenous ion can be estimated from flux of the ion (J_i) when there is no damage to the skin, that is, the ionic mobility of endogenous ion i (U_i) is constant at a constant voltage. The estimation of endogenous ion by constant voltage iontophoresis may be difficult, however. Figure 5 shows a schematic diagram of *in vivo* non-invasive sampling by iontophoresis. The potential gradient across the skin membrane cannot be measured precisely because the electrode cannot be used in the body. In fact, the detectable potential gradient of *in vivo* application is the sum of the potential gradient of the skin, the potential gradient in the body and the potential gradient of the skin, as shown in Fig. 5. The electric current through the membrane obeys the following relationship:

$$I_i = \Sigma [Z_i F C_i U_i E S], \qquad (10)$$

where I_i is the total current through the membrane, Z_i, F, C_i, U_i, E and S are, respectively, charge of ion i, Faraday constant, concentration of ion i, ionic mobility of ion i, potential gradient and surface area of membrane available for ion permeation. The electric current through the skin membrane increases when the concentration of ion i is increased and/or the concentration of another ion species increases at constant voltage. Skin damage is caused by too great an increase in electric current through the membrane [4]. The concentration of endogenous ion cannot be estimated when the ionic mobility is altered because of skin damage.

When the current is constant, the potential gradient (E) is inversely proportional to the concentration of ion i (C_i) because the flux of ion i (J_i) is constant at a constant current, as shown in equation (11):

$$E = (J_i/U_i)\ (1/C_i). \qquad (11)$$

This equation means that constant ionic mobility, which represents no damage to the skin, is required to estimate the concentration of endogenous ion using constant current iontophoresis. It is also necessary to measure precisely potential gradient across the skin. But the potential gradient cannot be measured precisely, as mentioned above, because it decreases when the concentration of ion i increases and/or the concentration of another ion species increases at a constant current. The increase in potential gradient should be minimized to reduce skin damage.

Using this method, the fraction of the flux of lactic acid over the total flux of lactate and chloride ion was constant when the potential gradient was changed as shown in equation (7). A minimum steady-state flux of lactate and chloride ion must be measured. A minimum constant current was applied to obtain the minimum steady state flux and this also reduced skin damage. It is easy to measure the current through skin at the outer side of the body (for example, in the device) because the current is constant on the circuit. Estimation of

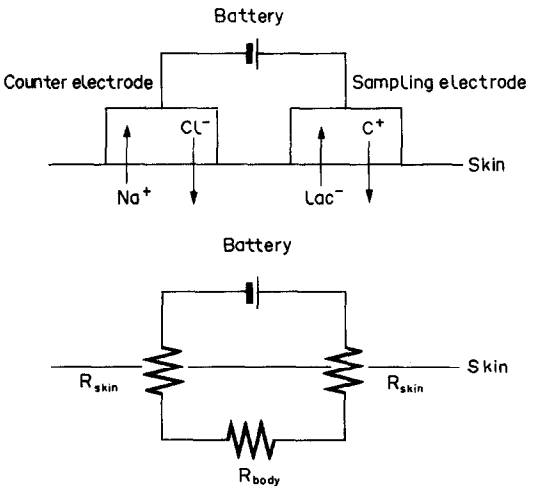

Figure 5
Schematic diagram of *in vivo* non-invasive sampling of endogenous ion by iontophoresis.

lactic acid concentration requires a constant ratio of ionic mobility of chloride and lactate (U_{Cl}/U_{lac}) [equation (8)]. The ratio of ionic mobility of the two compounds is inherently constant when there is no skin damage, and variation in the ionic mobilities is parallel when the skin is damaged. The U_{Cl}/U_{lac} ratio through damaged skin increases similarly to that through intact skin when using this new method. Further work on this non-invasive sampling method is necessary to understand this effect. Once this is resolved, the new method will be useful for determining an endogenous ion whenever skin has been damaged. The disadvantage of the method is that the concentration of lactic acid in the dermis side cannot be determined without steady-state fluxes of lactate and of chloride ion as an internal standard.

Conclusions

Non-invasive sampling of endogenous ion by iontophoresis using chloride ion as an internal standard is suggested to reflect the concentration of the ion in spite of changes in applied current and/or skin resistance.

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