Contribution of Bulk Flow to Transport Mechanisms of the Membranes Surrounding Amniotic Fluid in the Rabbit

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=ABSTRACT=

The objective of the present study is to assess the contribution of bulk flow to the regulatory mechanism of amniotic fluid volume and its ionic concentration in the membranes surrounding the amniotic fluid. For quantitative assessment, we prepared 4 kinds of artificial amniotic fluids (isotonic isovolumetric, hypotonic isovolumetric, isotonic hypervolumetric and hypotonic hypervolumetric ones) by replacing 70% of amniotic fluid of pregnant rabbits with water or normal Tyrode solutions. Isoosmotic saline of 0.5 ml volume containing 0.05% Congored and 15 mM/l LiCl was administered initially into amniotic sacs of all subject animals. Samples of amniotic fluid were collected in after 30 and 90 minute intervals; the concentrations of Congored, Na^+ and Li^+ were determined and compared.

Followings are the results obtained.

1. From isovolumetric and increased Congored group, we couldn't find significant change in Li^+ and Na^+ concentration in isotonic amniotic fluid. However, Na^+ concentration increased significantly as well as a striking increase in Congored concentration in hypotonic amniotic fluid.

2. In isovolumetric and decreased Congored group, the rate of $[Li^+]$ decrement and the rate of $[Na^+]$ increment were much higher in hypotonic amniotic fluid than in isotonic.

3. In hypervolumetric and increased Congored group, the rate of Na^+ efflux increased proportionately with the increment of Congored concentration up to 0.98, which was higher than the rate of Li^+ efflux in isotonic amniotic fluid. However, the increment of Na^+ concentraion was rather related with the initial Na^+ concentration in hypotonic amniotic fluid, showing inverse relationship. Li^+ concentration increased only when there was a marked increase in Congored concentration and approached near a maximum value or 1.

4. For hypervolumetric and decreased Congored group, the observations were identical to isovolumetric and decreased Congored group.

From these results the following conclusions could be made: 1) There is no net movement of water or monovalent cations across the membranes surrounding amniotic fluid in isotonic isovolumetric condition. In contrast, there is a net efflux of amniotic fluid by osmotic bulk flow, resulting in elevation of Na^+ concentration in hypotonic isovolumetric condition. 2) In hypervolumetric conditions, there is a massive efflux of amniotic fluid or solvent drag through the surrounding membranes by filtrative bulk flow, where the rate of Na^+ efflux has a linear relationship with that of water efflux. This is assumed to be carried out through enlarged and newly opened intercellular spaces resulting from increased intraamniotic pressure. 3) Once increasing intraamniotic pressure reaches a point allowing Li^+ to pass through during osmotic bulk flow in hypotonic amniotic fluid, Na^+ influx seems to occur by diffusion simultaneously or immediately thereafter, too.

Key words: Li^+, Na^+, Congored, Amniotic fluid, Bulk flow

INTRODUCTION

Most researchers agree that the volume and osmotic concentration of amniotic fluid is controlled largely via extrafetal pathway such as the membranes surrounding amniotic fluid, particularly through chorioamniotic membranes and vascular membrane of the umbilical cord (Ross et al, 1983; Tomoda et al, 1985; Gilbert & Brace, 1989).

At present, it is known that there is no voltage difference across amniotic membranes and no evidence for active membrane transport was found across these cells (Lind et al, 1971). Some experiments performed *in vitro* reported that transport in chorioamniotic membranes were achieved by osmotic bulk flow and diffusion through intercellular pores, which allowed passage of only small molecules less than M.W. 1,000 (Seeds, 1970; Tomoda et al, 1985).

We have recently reported that Li ions injected into maternal blood were detected in amniotic fluid of expired fetus in sizeable amounts and, conversely, Li^+ injected into amniotic fluid disappeared over a period of time without any procedure. Therefore, we suggested that the membranes surrounding amniotic fluid played an important role in the transport of electrolyte as well as that of water (Kim et al, 1990; Chang et al, 1993).

Movement of water and Li^+ through the membranes surrounding amniotic fluid was dependent on osmotic concentration of amniotic fluid (Chang et al, 1993), and affected greatly by prolactin (Sohn & Sung, 1988). Accordingly, movement of water and Li^+ through the membranes surrounding amniotic fluid was assumed to be achieved by osmosis and diffusion (Chang et al, 1993). However, since total volume secreted in the fetal urine was estimated one and half to two times as large as the volume swallowed (Tomoda et al, 1985; Morris & Boyd, 1988),

the volume of amniotic fluid was expected to rise temporarily by so much as about 50% (Tomoda et al, 1987), subsequently, raising intraamniotic pressure which would, in turn, trigger filtration to occur through the membranes surrounding amniotic fluid. But, reports focusing on the filtration of amniotic fluid had not yet been met, probably because some technical difficulties are expected in such experiments. On the other hand, fetal urine was reported to be hypotonic as 75 mEq /l in Na^+ concentration (Lind et al, 1971). Nevertheless, osmotic concentration of amniotic fluid is always maintained at a constant level within a narrow range. As amniotic membranes should serve as beds for regulating osmotic concentration of amniotic fluid, we expected that in addition to osmosis and diffusion, which have already been proved to contribute to the membrane transport *in vitro* conditions (Cittadini, 1977; Ross et al, 1983; Tomoda, 1985; Wallenburg, 1986; Gilbert & Brace, 1989), filtration should be included in the transport mechanism of *in vivo* conditions.

Here, we replaced part of the amniotic fluid with isotonic or hypotonic solution to make a 50% volume increase and determined concentrations of Congored, Na^+ and Li^+. By contrasting with control experiment, in which the volume of amniotic fluid was maintained normally, we tried to focus attention on the regulation mechanism of elevated amniotic volume in the transport of water and various solutes through the membranes surrounding amniotic fluid.

METHOD

Materials

Later stage pregnant rabbits were used as the experimental animals, which were divided into 4 main groups according to volume and osmolarity (tonicity) of amniotic fluid, i.e., isotonic isovolumetric, hypotonic isovolumetric, isotonic hypervolumetric and hypotonic

hypervolumetric groups.

Procedure

Animals were anaesthetized in a supine position by injecting pentobarbital sodium (Nembutal) through the marginal ear vein at a dose of 30 mg per kg. The carotid artery was catheterized and connected via a 3 way stop cock to the physiograph (Device MX 6) to monitor the blood pressure. The jugular vein was also catheterized and infused with 150 mM NaCl solution by constant speed infusion pump.

Victims were incised in the abdomen; the uterus was exposed; 5 fetuses were numbered 1 to 5 in turn beginning from right side and injected into each amniotic cavity with 0.5 ml isoosmotic saline solution containing 15 mM LiCl and 0.05% Congored. Amniotic fluid volume of No. 1 fetus was used as control. Amniotic fluids of No. 2 and 3 fetuses were replaced with normal Tyrode solution and those of No. 4 and 5 fetuses were replaced with distilled water in 70% volume we-made isovolumetric group by exchanging exactly the same amount and, hypervolumetric one by replacing with more amounts so as to mark 1. 5-fold increase. Then, after 30 minutes we extracted amniotic fluid from No. 2 and 4 fetuses; after 60 minutes since then, i.e., at time 90 minutes, we extracted amniotic fluid from No. 3 and 5 fetuses.

Measurement of concentrations of Congored, Li$^+$ and Na$^+$

The concentration of Congored was determined from 1 ml fluid extraction with a colorimeter (Corning 253), using normal rabbit plasma as diluent. The concentrations of Na$^+$ and Li$^+$ were determined in the remaining amniotic fluid using the IL 943 Automatic Flame Photometer (Allied Instrumentation Laboratory).

Data analysis

Percentage change, Y (relative percentage increased or decreased) of concentration of X was calculated as follows:

$$Y = \frac{X_{30min} - X_{90min}}{X_{30min}} \times 100$$

where, X_{30min}: concentration of X in amniotic fluid at 30 minutes

X_{90min} : concentration of X in amniotic fluid at 90 minutes

X: Congored, Li$^+$, Na$^+$

Y: CR, Li, Na in case X=Congored, Li$^+$, Na$^+$ respectively.

Li$^+$(or Na$^+$) movement rate was calculated as

$$\frac{CR - Li(or\ Na)}{CR}$$

As Congored concentrations didn't show uniform results they - either increased or decreased over a 60 minute period- we divided data into increased Congored group and decreased Congored group. In isovolumetric group, percentage change of [Li$^+$] and [Na$^+$] determined in hypotonic condition was compared with those determined in an isotonic condition. In hypervolumetric group, the relationship between percentage change of Congored and Li$^+$ (or Na$^+$) was plotted on a graph, pursuing the correlation between isotonic and hypotonic conditions.

The comparison between the values determined in each time was evaluated using an unpaired t-test. A difference of P<0.05 was considered statistically significant.

RESULTS

Concentration changes of Li$^+$ and Na$^+$ in isovolumetric amniotic fluid

Data of increased Congored concentration: Congored concentration increased in 5 cases among a total of 8 cases, in which we replaced 70% of amniotic fluid with normal Tyrode solution. The rate of Congored concentration increment in this group was 8.0±1.98 %, showing great variation of the values. Though Li$^+$ concentration was decreased by 4. 0±4.04% and Na$^+$ concentration was increased by 1.4±2.45% from the initial concen-

Table 1. Percent changes of lithium and sodium concentration during increment of Congored concentration in isonatremic and hyponatremic amniotic fluid

Amniotic fluid	Initial[Na$^+$] (mEq/l)	Congored (%)	Li$^+$ (%)	Na$^+$ (%)
isonatremic (5)	137.3 ± 7.77	8.0 ± 7.98	1.4 ± 2.45	
hyponatremic (5)	101.3 ± 8.60	39.5 ± 9.52*	15.2 ± 10/87**	

About 70% of amniotic fluid were replaced with normal tyrode solution or distilled water. 0.5 ml of isosmotic saline containing 0.15 mM LiCl and 0.05% Congo red was introduced into each amniotic cavity. Initial Na$^+$concentration was measured 30 minutes after mixed fluid injection. percent changes were calcu- lated by {conc.(90 min) -conc.(30 min)} /conc.(30 min)×100. Asterisks indicate *P<0.005 and **P<0.005 which differ significantly from amniotic fluid.

Table 2. Percent changes of lithium and sodium concentration during decrement of Congo red concentration in isonatremic and hyponatremic amniotic fluid

Amniotic Fluid	Initial[Na$^+$] (mEq/l)	Congored (%)	Li$^+$ (%)	Na$^+$ (%)
Isonatremic (3)	141.3 ± 0.63	−44.8 ± 9.16	−2.8 ± 0.65	3.3 ± 1.06
Hyponatremic (5)	87.5 ± 15.00	−33.2 ± 12.33	−25.4 ± 4.46*	34.2 ± 9.72*

Note: See Table 1.

tration of 137.3 ± 7.77 mEq/l they had no statistical significance due to severe variation.

Among another 8 cases, in which we replaced 70% of amniotic fluid with distilled water, 3 cases showed a decrease in Congored concentration. The rate of Congored concen- tration increment was 39.5±9.52%, which was considered remarkably high and was viewed as significant in spite of great variation (P<0.005) compared with the former value. Li$^+$ concentration decreased by 0.9±2. 67%, which was statistically insignificant. How- ever, Na$^+$ concentration increased significantly by 15.2±10.87% during a 60 minute period from the initial concentration of 101.3±8. 60 mEq/l (0.025<P<0.05) (Table 1).

From these data we assumed that during solvent drag out of the amniotic sac in hy- potonic amniotic fluid either Na$^+$ efflux was restricted or Na$^+$ diffusion took place into the sac and reduced the concentration difference of Na$^+$.

Data of decreased Congored concentra- tion: Decrease of Congored concentration in amniotic fluid was attributed to urination and /or swallowing of the fetus. As the volume of urination and swallowing couldn't be known in this experiment the rate of Congored concentration decrement had no meaning. In isotonic and decreased Congored group, which include 3 cases, Li$^+$ had a slight decreasing tendency, and Na$^+$ had a slight increasing tendency. However, in hypotonic and decreased Congored group, which include 5 cases, Li$^+$ decreased by 25.4±4.46% and Na$^+$ increased by 34.2±9.72%. These were very high values compared with 2.8±0.65 and 3.3 ±1.06 of isotonic group, respectively (P<0. 005) (Table 2).

It may be that the increase of Na$^+$ con- centration and decrease of Li$^+$ concentra-tion in hypotonic amniotic fluid can be attributed to Na$^+$ influx by diffusion and Li$^+$ efflux, even if it is taken into consideration that

Table 3. Percent changes of sodium concentration during increment of Congo red concentration in isonatremic and hyponatremic hydramnios

Ammiotic Fluid	Initial[Na$^+$] (mEq/l)	Congored (%)	Na$^+$ (%)
Isonatremic (5)	138.4±11.74	32.3±19.01	3.4±2.06
Hyponatremic (5)	87.0±5.90	51.8±21.12	23.0±17.94*

About 70% of amniotic fluid were withdrawed and then produced 150% hydraminios by instilling normal Tyrode or distilled water. Asterisk indicates P>0.005
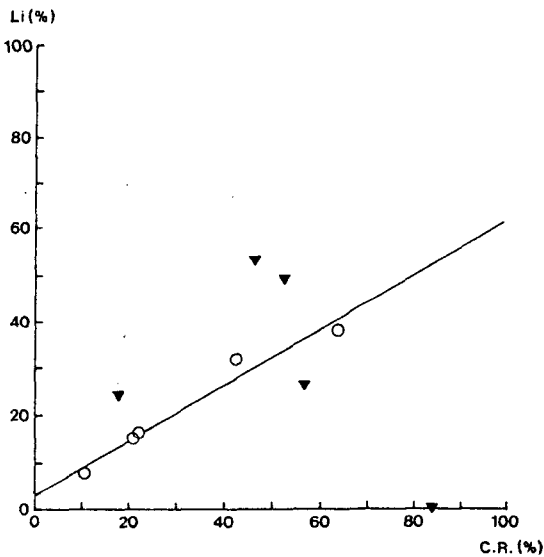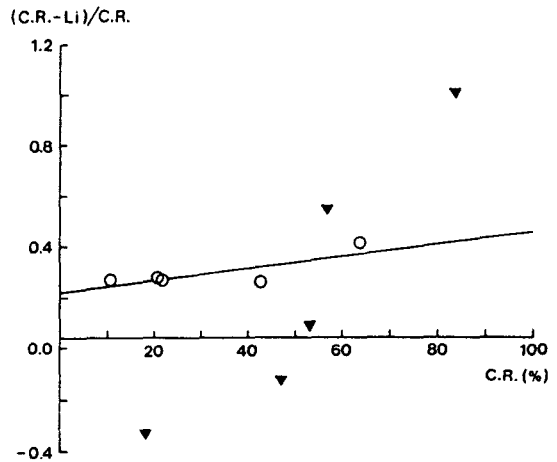

Fig. 1. Percent change of lithum ion concentration in relation to increment of Congo red concentration in isonatremic and hyponatremic hydramnios. Percent changes of Li$^+$ and Congo red(CR) during the period of 30-90 minutes after intraamniotic injection were calculated by {conc.(30 min)-conc.(90 min)}/conc.(30 min)×100. Percent changes of Li$^+$ concentration in isonatremic(○) and hyponatremic(▼) 150% hydramnios were plotted against the percent changes of Congo red concentration. For the regression of isonatremic hydramnios. γ=0.982, P< 0.005.


Fig. 2. Estimated rate of lithium movement along with water into or out of amniotic sac in relation to increment of Congo red concentration in isonatremic and hyponatremic hydramnios. Estimation of rate of Li$^+$ movement along with water calculated by (Increment of Congo red conc.-Increment of Li$^+$ conc.)/(Increment of Congo red conc.)

Estimated Li$^+$ movement rates (CR-Li)/CR during the period of 30-90 minutes in isonatremic(○) and hyponatremic(▼) hydramnios were plotted against the percent changes of Congo red concentration {CR(%)}. For the regression of isonatremic hydramnios, γ=0.790, P < 0.075
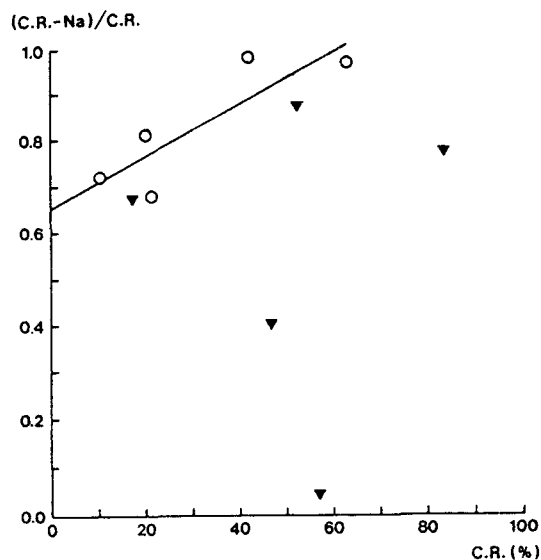
there was fetal urination into amniotic fluid.

Concentration changes of Li$^+$ and Na$^+$ in hydramnios

Data of increased Congored concentration: Increase of Congored concentration during a 60 minute period was 32.2±19.01% in isotonic amniotic fluid and 51.8±21.2% in the hypotonic one. Though the latter appeared larger than the former, this observation was statistically insignificant (Table 3).

Changes of Li$^+$ concentration: The relation
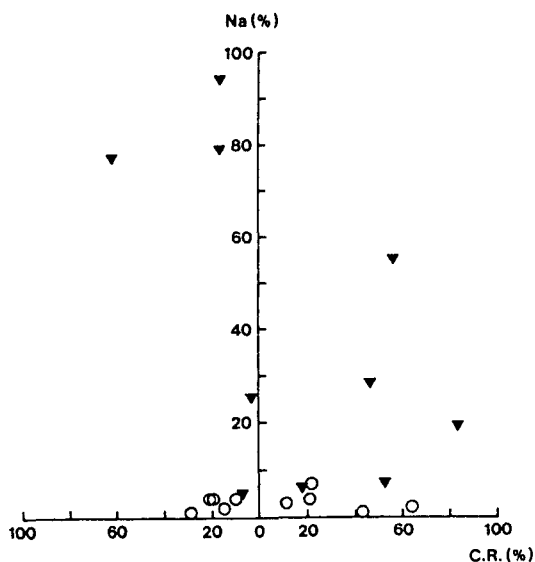
Fig. 3. Percent changes of sodium ion concentration in relation to increment or decrement of Congo red concentration in isonatremic and hyponatremic hydramnios. Percent changes of [Na+] in amniotic fluid during the period of 30–90 minutes after 150% volume challenge were plotted against the percent changes of Congo red concentration. Regardless of whether Congo red concentrations were rise or fall, [Na+] in isonatremic hydramnios(○) were within basal limit, but in hyponatremic hydramnios(▼), markedly higher increase were observed.

Fig. 4. Estimated rate of sodium movement along with water out of amniotic sac in relation to increment of Congo red concentration in isonatremic and hyponatremic hydramnios. Refer to Fig. 2 & 3. For the regression of isonatremic hydramnios(○), $\gamma=0.861$, $P<0.05$.

between the rates of [Congore] and [Li+] increment was illustrated in Figure 1. Percentage change of Li+ concentration had increased linearly with that of Na+ concentration in isotonic amniotic fluid (r=0.982, P <0.05). But a regular trend couldn't be found in hypotonic conditions. As shown in Figure 2, Li+ movement rate had been confined within the range of 0.26 to 0.28, while percentage change of Congored increased up to 43%. However, it became 0.41 when percentage change of Congored reached at 64 % in isotonic amniotic fluid (r=0.790, P<0. 075). In hypotonic amniotic fluid, Li+ movement rate had no observable trend until percentage change of Congored concentration

reached 53%, and then began to increase abruptly from 57% and reached 1 at 84%.

From these results we could realize that only small amounts of Li+ in amniotic fluid passed through the membranes by solvent drag in isotonic hydramnios. In contrast, as shown in Figure 1 that represented osmotic bulk flow in hypotonic hydramnios, increased intraamniotic pressure initially allowed only water molecules with a few Li+ to pass through. But, as the pressure increased and reached certain point where intercellular space became large enough to pass Li+ out, it allowed large amounts of Li+ to pass through by solvent drag.

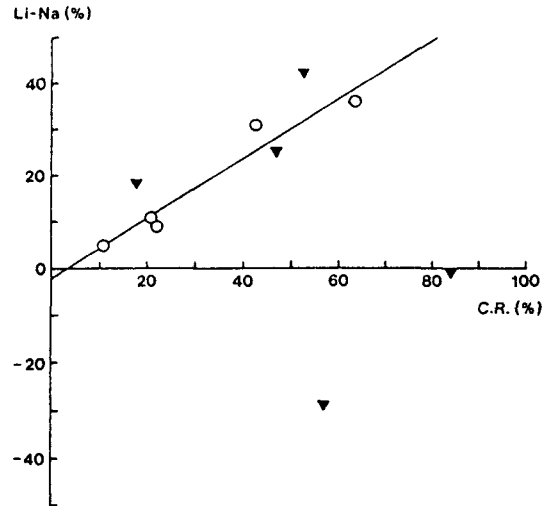**Changes of Na+ concentration**: In isotonic hydramnios Na+ concentration was 138.4±11. 74 mEq/L at first and increased by 3.4±2.06% (Table 3). Though percentage changes of [Congored] and [Na+] did not appear to have any relationship (Figure 3), Na+ movement
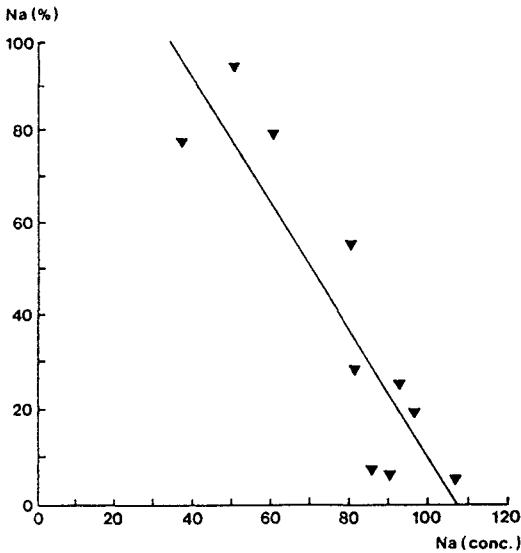
Fig. 5. Percent increase of sodium ion concentration in relation to it's initial concentration in hyponatremic hydramnios. Percent increase of [Na⁺] during the period of 30-90 minutes was calculated by {[Na⁺]90 min - [Na⁺]30 min}/[Na⁺]30 min × 100. Percent increase of [Na⁺] correlated negatively with it's intial concentration. For the regression, γ = 0.890, P<0.05.

Fig. 6. Scatter for the difference of percent changes between sodium and lithium ion concentration in relation to increment of Congo red concentration in isonatremic and hyponatremic hydramnios. Refer to Fig. 1 & 3. Differences correlated positively with Congo red increment. For the regression, γ=0.890, P<0.05

rate became so much higher, as percentage change of [Congored] increased, that it reached 0.98 at 43% (r=0.861, P<0.05) (Figure 4). This value was the one that closely approached 0.04, which was obtained at *in vitro* experiment. Therefore, high pressure due to excess of amniotic fluid in isotonic condition was supposed to raise Na⁺ efflux ratio in solvent drag by enlargement of intercellular spaces in the membranes.

In hypotonic hydramnios Na⁺ concentration was initially 87.0±5.90 mEq/; and increased by 23.0±17.94%. This seemed remarkably high compared with the value of isotonic hydramnios (P<0.005), and had no significant relation with percentage change of [Congored] (Figure 3). Having considered the Li⁺ data, we assumed that, while the degree of pressure increase was still low, water molecules were the only component capable of flowing out

and, once it became high enough for intercellular spaces to allow bigger molecules to pass through, Na⁺ would be dragged out with Li⁺ by filtrative bulk flow. However, by the fact that Na⁺ concentration increased in all cases and its rate of increase was inversely related to the initial concentration (r=0.89, P <0.005) (Figure 5), we could propose that sizable amounts of diffusion contributed to the transport mechanism.

Comparison between percentage changes of [Li⁺] and [Na⁺]: ΔLi-ΔNa was regarded as nearly equal with ΔLi, because ΔNa was negligible in isotonic amniotic fluid . But, since ΔNa was also increasing following ΔLi, the rate of Li⁺ dragged out by filtrative bulk flow was estimated to be low (Figure 6, Figure 1). In hypotonic amniotic fluid, of which percentage change of [Congored] was high, Na⁺ concentration increased even if

Table 4. Percent changes of sodium concentration during decrement of Congored concentration in isonatremic and hyponatremic hydramnios

Amniotic fluid	Initial[Na$^+$] (mEq/l)	Congored (%)	Li$^+$ (%)	Na$^+$ (%)
Isonatremic (5)	142.2 ± 8.80	−19.6 ± 6.36	−27.8 ± 22.72	3.0 ± 1.27
Hyponatremic (5)	69.9 ± 26.01	−20.2 ± 20.92	−34.8 ± 29.25	56.0 ± 34.57

See table 3.

percentage change of [Li$^+$] was near 0 (Figure 1, Figure 3). So, transport of monovalent cations appeared to be by solvent drag and by diffusion during or immediately after solvent drag. In addition, Li$^+$ efflux seemed to be influenced to a larger degree by Na$^+$ influx (Figure 5) than by percentage change of [Congored] (Figure 2).

Data of decreased Congored concentration: The rate of Congored concentration decrement was remarkably lower in hydramnios (Table 4) than in isovolumetric amniotic fluid (Table 2), indicating that more amniotic fluid flowed out of the membranes under the same conditions.

In isotonic amniotic fluid the rate of Li$^+$ concentration decrement far exceeded that of Congored concentration, indicating that Li$^+$ flowed out by solvent drag and also by diffusion, which was implied by an increase of Na$^+$ concentration.

In hypotonic amniotic fluid the rate of Congored concentration decrement was similar to that in isotonic amniotic fluid; the rate of Li$^+$ concentration decrement was slightly higher than that of Congored; Na$^+$ influx was noteworthy, that is, the rate of [Na$^+$] increment, which was higher than in isotonic amniotic fluid (P<0.005), was very prominent feature. So, it might be said that the rate of Na$^+$ concentration increment was inversely related to its initial concentration, as was the case in increased Congored concentration. But we couldn't evaluate the relationship between Congored and Na$^+$ properly, because we did not know the volume and concentration of fetal urine.

DISCUSSION

The volume and concentration of fetal amniotic fluid are maintained at a constant level in spite of fluctuation of its volume and concentration by fetal urination, which is known to be hypotonic, and by swallowing of isotonic amniotic fluid (Lind et al, 1971; Tomoda et al, 1985). Thus, it was suggested that amniotic fluid is controlled through a extrafetal pathway such as chorioamniotic membranes in addition to a fetal pathway (Ross et al, 1983; Tomoda et al, 1985). According to some in vitro experiments, chorioamniotic membranes allowed the passage of only small molecules less than 1,000 in molecular weight during bulk flow and/or diffusion (Seeds, 1970) and, Li$^+$ was assumed to be transported in considerable amounts through the membranes surrounding amniotic fluid, since the rate of Li$^+$ disappearance determined in expired fetus was about half levels of living fetus (Kim et al, 1990). But, from the observations that amniotic fluid in expired fetus showed a great reduction in volume and showed a large difference from Na$^+$ metabolism in the living fetus(Cittadini et al, 1977; Tomoda et al, 1985; Canning & Boyd, 1984), above results obtained from expired fetus should be cautiously applied to the study of amniotic regulatory mechanism of the living fetus.

In this experiment we initially administered Congored and Li$^+$ into the amniotic sac and determined the concentrations changes. The

change of Congored concentration was used as a yardstick reflecting whether or not there was urination. If Congored concentration was either increased or maintained steady, we considered there was no urination and tried to investigate the nature of transport mechanism. If Congored concentration was decreased we considered it as a sign of urination during the experimental period and tried to draw some possible explanation.

Among the isotonic group Li^+ and Na^+ concentration didn't show many changes in cases of increased Congored concentration, which meant decrease of amniotic fluid volume. This might be attributed to the near absence of intercellular pores already turned open in chorioamniotic membranes and, consequently, small amounts of bulk flow occurred through them, driven by an osmotic pressure of 27 mOsm (Canning & Boyd, 1984). In the hypotonic group, increase of Congored concentration was higher than in the isotonic group. So, more osmotic pressure was thought to be imposed through the pores. The large increase of Na^+ concentration was noteworthy and was attributed to either relative reduction of Na^+ efflux by solvent drag or Na^+ influx by diffusion. In this case, diffusion seemed more likely, which occurred simultaneously or immediately after bulk flow, since no change of Li^+ concentration was observed (Kim, 1991).

In decreased-Congored group Li^+ and Na^+ concentrations in isotonic conditions appeared to have a tendency to either decrease or increase, depending on the situation, however, they had no special significance just as in the isotonic and elevated-Congored group. In hypotonic condition, decline of Li^+ concentration and elevation of Na^+ concentrat-ion was such a remarkable feature that we could presume that elevated intraamniotic pressure imposed by fetal urination enlarged existing intercellular spaces and opened new ones, and, as a result, further osmotic bulk flow occurred to the outside of the membranes and also by diffusion, to the inside.

As reported before in some *in vitro* experiments (Cittadini et al, 1977; Tomoda et al, 1985), amniotic membrane was 400-600 μm thick, diffusion rate of water through intercellular pores was 5-fold slower than in solution, and diffusion rate of Na^+ was less than water by the third power of 10. In addition, it was reported that amniotic membrane allowed passage of water-soluble molecules less than M.W. 1,000 (Seeds, 1970), and that the volume transported by bulk flow was several and a hundred times as large as the volume transported by diffusion in chorionic and amniotic membrane, respectively (Lind et al, 1971).

On the contrary, no detailed reports have been found about transport property under *in vivo* conditions, except evidence supporting the presence of transport of water and Na^+ (Canning & Boyd, 1984) and our previous report (Kim, 1991), where it was implicated that osmotic bulk flow might be followed by diffusion, since Na^+ concentration of amniotic fluid recovered to normal after alteration of its concentration.

In this experiment, elevation of intraamniotic pressure made by increments of volume to 1.5 times normal pressure did not produce uniform results; Congored concentra-tions were sometimes elevated and sometimes decreased. However, decrement of Congored concentration represented the presence of fetal urination with or without swallowing since Congored had the ability to combine with proteins in amniotic fluid and not to pass through the membranes surrounding amniotic fluid.

The result obtained under isotonic conditions showed a similar pattern to the control, in which Congored and Li^+ concentration decreased in parallel, and Na^+ concentration increased a little, whereas, in hypotonic conditions, the rate of Li^+ concentration decrement exceeded that of Congored, indicating Li^+ flowed out in considerable amounts during bulk flow. In addition, a remarkable increase in Na^+ concentration, which was inversely related with initial concentration, implied that

diffusion also occurred in sizeable amounts. But the results obtained in decreased Congored concentrations couldn't be interpreted properly because they had unknown factors such as volume of fetal urine, volume of fetal swallowing, and Na^+ concentration of fetal urine.

Increase of Congored concentration among amniotic fluid was thought to mean decrease of amniotic fluid volume. If only water molecules passed through the membrane, Li^+ concentration of amniotic fluid would increase at the same rate with Congored, and if Li^+ passed through the membrane following water flux its concentration wouldn't be changed.

In hypervolumetric and isotonic group, Li^+ concentration increased as Congored concentration increased. The amount of Li^+ efflux was 20% of water and 40%, in case the rate of Congored concentration increment was as high as 60%. These were estimated to be very low when compared with Congored efflux. Therefore, it could be said that the intercellular spaces of amniotic membrane allowed passage of Li^+ with some restrictions.

In contrast, the degree of Na^+ concentration increment was so small in all cases that most Na^+ seemed to flow out of membrane with water. The more Congored concentration increased in amniotic fluid, the higher the degree of Na^+ efflux was. So, it had become 97% when the rate of Congored concentration increment reached 40%. This result was coincident with the report that the reflection coefficient of Na^+ was 0.02 at chorioamniotic membranes during bulk flow in vitro (Seeds, 1970).

If increased intraamniotic pressure caused by volume challenge to amniotic fluid forced intercellular pores to open or expand, more water would flow out through them, however, Na^+ efflux would be restricted at this first stage. As the pores became large enough to allow Na^+ out, it would be free to flow out with water. However, there was a big difference between the efflux rate of Li^+ and Na^+. Though smaller than Na^+ as an atom, Li^+ is similar in size to Na^+ as an ion in

solution. Therefore, from a physical viewpoint, the same amounts should be transported. However, this is unlikely to be true. Li^+ transport at the membrane of erythrocyte was slower than Na^+ transport (Tosteson, 1981). In this case it was explained that Li^+ transport was slowed down, since Li^+ is not a normal constituent of body fluid and, subsequently, its movement would be restricted by the cell membrane. Conditions would be dependent on intracellular and extracellular environment. In the renal proximal tubule the rate of reabsorption of Li^+ was similar to that of Na^+ (Hayslett & Karshgarian, 1979), while it was 70% levels of Na^+ levels in the thick ascending limb of Henle loop (Herbert & Andreoli, 1984). From these reports we might infer by analogy that the lower rate of Li^+ efflux could be attributed to electrochemical factors as was true in the thick segment of Henle loop. Anyway, what we could say here is that there is a slow Li^+ efflux, since Li^+ administered into maternal blood vessel appeared in amniotic fluid and, after a period, became rather higher in concentration than in maternal blood (Shim & Sung, 1987).

When amniotic fluid was replaced with a hypotonic one, the rate of Congored concentration increment was higher than that in isotonic fluid, just as in the control data. In this case, osmotic pressure difference plus filtration force could be regarded as contributing factors. In cases where the rate of Congored concentration increment was low, it was similar to that of Li^+ concentration. This implied that Li^+, just as if Congored, couldn't pass through the membrane because of small size of intercellular pores for these cations, and, subsequently, the transport was mainly driven by osmosis of water. In cases that the rate of Congored concentration increment was high, Li^+ concentration was unchanged. This implied, as stated before, that the pore size permitted Li^+ to pass through by solvent drag. On the other hand, Na^+ concentration showed a tendency to increase in spite of severe individual variation in hypotonic cases. That

is, the rate of Na^+ disappearance or (CR-Na) /CR was much lower than in isotonic cases. The same principle could be applied to Li^+, other than that, after expansion of pores was enough to permit Na^+ to pass through the membrane, there would be influx of Na^+, because it is a normal component in the fluid outside the membrane. Seeds (1970) reported that contribution of diffusion to the transport mechanism of chorioamniotic membranes was estimated to be small compared to that of bulk flow, and Kim (1991) reported that Na^+ transport was carried out at first by bulk flow and, then, accompanied by diffusion in later stage of bulk flow.

Therefore, we could speculate as follows. If the volume of amniotic fluid became increased as a result of fetal urination minus swallowing, resulting elevation of intraamniotic pressure would activate filtration. While the degree of elevation of the pressure was still low, mostly solvent or water would be filtered out. But if the pressure increased enough to make intercellular pores allow such solutes as Na^+ to pass through, it would also escape with water through the membrane by solvent drag. Furthermore, since fetal urine was known to be hypotonic, even if filtrative pressure increment was too small to evoke filtration of Na^+, osmotic pressure difference would drive water to flow out through the membrane.

Despite the *in vitro* evidence in support of osmotic bulk flow in the transport of water and Na^+ across the membranes surrounding amniotic fluid (Seeds, 1970), debates on the presence of osmotic bulk flow have continued in *in vivo* experiments: one groups confirmed it (Ross et al, 1983; Gilbert & Brace, 1989) and the other group denied the presence of bulk flow(Ervin et al, 1986). In this regard, our studies support the notion of the former group, and suggest that the transport of water is achieved by both filtrative and osmotic bulk flow, and that the amount of solvents dragged through by water during this process, is determined by the number and size of inter-

cellular pores recruited.

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