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Effect of Changing Amniotic Fluid Osmolarity on the Li^+ Transport Through the Membrane Surrounding Amniotic Fluid in the Rabbit

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= ABSTRACT=

To study the regulation of amniotic fluid volume and electrolyte concentration by the membranes surrounding the amniotic fluid, the rate of Li^+ disappearance from amniotic sac of expired fetuses were examined while increasing the amniotic volume and osmolarity in rabbits. After intraamniotic injection of 1 ml isosmotic saline (about 20% of the amniotic fluid volume) containing 15 mM LiCl and 0.5 g/L Congored, the time courses of Li^+ and Congored disappearance were determined. From there the Li^+ clearance through the extrafetal routes was estimated and compared with that obtained from living fetuses. The volume, Na^+ concentration and osmolarity of amniotic fluid were measured and their relationships with Li^+ disappearance were evaluated.

The following results were obtained:

1. The rate of disappearance from amniotic fluid of living fetuses during the first 30 minutes was strikingly higher for Li^+ than for Congored, suggesting that extrafetal routes exist. At 60 and 90 minutes, however, the disappearance rate of Li^+ was less than that of Congored, suggesting the possibility of Li^+ reentry through fetal urination.

2. The disappearance of Li^+ from the amniotic fluid of the expired fetus was substantial, although lower than that of living fetuses, throughout the experimental period.

3. The Na^+ concentration and the osmolarity of the amniotic fluid of expired fetus measured 30 minutes after an intraamniotic injection of isoosmotic saline showed wide variation, but thereafter they changed gradually towards the normal extracellular fluid level.

4. When the amniotic fluid was iso- or hyposmolar, the rate of Li^+ disappearance from the amniotic fluid of the expired fetuses showed little variation. However, when the amniotic fluid was hyperosmolar, the rate at 30 minutes was markedly lower than those of isosmotic or hyposmotic amniotic fluid. At 90 minutes, the rate of Li^+ disappearance in hyperosmolar fluid reached a similar level to the rate in isosmolar fluid.

5. The intraamniotic injection of 400 mOsm/L saline solution decreased the disappearance rate of Li^+ from expired fetuses, while the injection of mannitol into the maternal vein induced no significant change.

From these results it is concluded that: 1) a significant amount of Li^+ may leave the amniotic fluid via filtration through the membranes surrounding the amniotic fluid, 2) during hyperosmolar challenge to amniotic fluid, osmotic bulk flow might counteract the filterable loss, and 3) Li^+ disappearance might continue even after the volume and osmolarity of the amniotic fluid have recovered to control values.

Key Words: Li^+, Amniotic fluid, Osmolar concentration

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INTRODUCTION

The regulation of amniotic fluid volume is increased through fetal urination and pulmonary secretions and decreased by fetal swallowing. This suggests a net positive balance, as the volume of swallowing is about a half (Paul et al, 1956; Tomoda et al, 1985; Morris & Boyd, 1988) or three quarters (Friedman et al, 1956) of the volume of excretion. However, the regulation of its osmolarity suggests a net negative balance since small isosmotic swallowing could not replace the hyposmotic urination. However, fetal amniotic fluid continues to maintain its volume and osmolarity within certain normal range. From this fact, it has been supposed that there should exist a extrafetal regulation pathway (Ross et al, 1983; Brace, 1986) and that this pathway would be via the membranes surrounding amniotic fluid, in particular the amnion and chorion laeve of placenta(Tomoda et al, 1985).

The amniotic membrane either extrudes water from amniotic fluid or controls the influx or efflux of solutes in order to compensate for the hypoosmolarity of fetal urine. Correspondingly, it has been reported that water leaves the amniotic fluid and ions enter (Brace, 1986). The diffusion rate of water through amnion and chorion laeve, which are 400~600 μm thick, is one fifth of that measured in solution (Tomoda et al, 1985). Also the water transport rate by osmotic bulk flow is several times faster than diffusion at the amnion laeve and even a hundred times faster at the chorion laeve (Seeds, 1970). As the amniotic fluid is normally lower by 27 mOsm/L in osmolarity and by 13 mEq/L in Na^+ concentration than maternal plasma (Canning & Boyd, 1984), water can move out of it. However, the amount of water movement is believed to be very small because the osmotic reflection coefficient for NaCl is only 0.02 (Seeds, 1970).

Little is known about the transport of solutes through amniotic membrane. In spite of blockage of the placental pathway maternal Na^+ is found with 10% level in fetal plasma (Canning & Boyd, 1984) and drugs such as ampicilline (Seeds, 1980) and dehydroepiandrosterone sulfate (Ylikorkala et al, 1979) introduced into amniotic fluid of expired fetus were also found in maternal plasma. These facts suggest that substances with small molecular weight or low lipid solubility could pass through the amniotic membrane in any direction, although those substances with molecular weights above 1,000 such as mannitol were unable to pass through. The reflection coefficients of urea, NaCl and glucose are 0.01, 0.02 and 0.05, respectivley (Seeds, 1970). As the potential difference for sodium and lithium ions across the membrane is very small (Lind et al, 1971) the possibility for the active transport of these ions can be disregarded. So, the paracellular pathway is considered to be the only channel capable of transporting sodium and lithium ions (Seeds, 1970). In the case of the expired fetus, the rate of water transport was about half of that in the living fetus (Paul et al, 1956; Tomoda et al, 1985) and one tenth of that in water for Na^+ (Cittadini et al, 1977; Tomoda et al, 1985). Other than diffusion, little is known about the transport of solute through amniotic membrane. Fetal diuresis into amniotic fluid did not bring about polyhydramnios (Brace, 1986), therefore, excess amniotic fluid was believed to be drained rapidly by fetus or through amniotic membrane or both. The slowness of Li^+ appearance from maternal plasma into amniotic fluid in the expired fetus, only half the rate of that in the living fetus, suggested the presence of a sizable Li^+ influx through another route than the fetus itself (Kim et al, 1990). However, the rate of Li^+ disappearance from amniotic fluid in living fetus was dependent upon the Li^+ concentration (Kim et al, 1990). This could be partly explained as Li^+ flowing back into the amniotic fluid through the fetal urine since a higher blood level of Li^+ would necessarily result in a greater excretion of Li^+ through the urine (Thomsen & Leyssac, 1986). Concentration-de-

pendent diffusion could also be partly explained if bidirectional transport were possible for Li^+ as is the case in Na^+ transport (Canning & Boyd, 1984). It seems that bidirectionality of Na^+ transport and other cations may contribute to the regulation of volume and osmolarity of the amniotic fluid by allowing the efflux of these cations in hyperosmolar condition.

As fetal urine is hypoosmolar and its volume is larger than swallowing volume, most part of recent studies on the amniotic fluid have focused on water disappearance (Ross et al, 1983; Lotgering & Wallenburg, 1986) and ionic influx (Canning & Boyd, 1984), rather than ionic efflux. The mechanisms known up to now of water disappearance across the membrane are osmotic bulk flow and diffusion (Seeds, 1970; Ross et al, 1983). The other possible pathway is filtration driven by intraamniotic pressure (Gilbert & Brace, 1989). Therefore, the solutes extruded following water efflux in the process of osmotic bulk flow or solvent drag could also be extruded in large quantity by filtration in degrees dependent on the osmolar concentration.

To test the idea that water and solutes are filtered through amniotic membrane by amniotic volume challenge, we injected isoosmotic saline solutions including Li^+ into the amniotic fluid of pregnant rabbits and recorded the time course of Li^+ disappearance in relation to osmolarity. This was then analyzed by raising osmolarities of either amiotic fluid or maternal plasma to investigate the relation between filtration and osmotic bulk flow that is cooperative or antagonistic depending on conditions.

METHODS

Materials

Later stage pregnant rabbits were used as experimental animals. Among them 24 rabbits finished full procedure and another 5 rabbits were used for supplementary experiments.

Injection of LiCl and Congored

Animals were anesthetized 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 using a constant speed infusion pump. 30 to 60 minutes after the NaCl infusion, the uterus was exposed and 3 fetuses were incised at amniotic membrane, tied at umbilical vessels, taped with tissue adhesive (histoacryl, Braun Melsugen AG Co.), and numbered 1 to 3 from the right side expired fetus group. The next 3 fetuses were numbered 4 to 6 and classified as the living fetus group. Each fetus was injected into amniotic fluid with 1 ml solution containing 15 mmole/L LiCl, 135 mmole/L NaCl and 0.5 gm/L Congored as a volume challenge.

Injection of hyperosmolar solution

To study the effect of amniotic fluid osmolarity on the Li^+ disappearance, supplementary experiments were conducted. In the same animals, hyperosmolar solution which contained 15 mmole LiCl plus 185 mmole NaCl, was injected into amniotic fluid. In other animals, 20% mannitol (20 ml per kg) was infused into maternal vein for 10 minutes, 20 minutes before the injection of LiCl solution into amniotic fluid. The rest of the experimental procedure was identical in all groups.

Measurement of Congored concentration and calculation of disappearance rate and amniotic fluid volume

After volume challenge, the amniotic fluid was extracted from the first and fourth fetuses at 30 minutes, from the second and fifth at 60 minutes and from the third and sixth at 90 minutes. The concentration of Congored was measured from 1 ml fluid extraction with a colorimeter (Corning 253), using normal rabbit plasma as standard solution and diluent. In living fetuses the concentration of Congored was plotted on semilog paper and the zero time con-

centration was estimated by extrapolating the straight portion of the curve. Calculation of disappearance constant and Congored disappearance rate was as follows:

Congored disappearance rate (%/t)
$$=(1\text{-}e^{-kt})\times 100$$
where, disappearance constant (K)
$$=\frac{2.303}{t}\times\log\frac{C_o}{C_t}$$
t: time
C_o: concentration at time zero
C_t: concentration at time t

Amniotic fluid volume was calculated at each time in expired fetuses according to diluting principle, assuming that congored did not leave the amniotic space.

Measurements of Na^+, Li^+, and osmolarity

The concentrations of sodium and lithium ions in amniotic fluid were measured using the IL 943 Automatic Flame Photometer (Allied Instrumentation Laboratory) and the Li^+ disappearance rate was calculated as follows:

$$Li^+ \text{ disappearance rate } (\%/t)=\frac{D_i-(V_a\times C_t)}{D_i}\times 100$$
where, D_i: injection dose
V_a: amniotic fluid volume
C_t: concentration at time t

Osmolarity

The osmolarity of amniotic fluid was measured using an osmometer (Advanced Instrument 3w).

Statistical analysis

The differences between Congored and Li^+ disappearance rates in living fetus, the differences of Li^+ disappearance rate between living and expired fetuses and the comparison among values in each time were evaluated using an unpaired t-test. A difference of $P<0.05$ was considered statistically significant.

RESULTS

Disappearances of Li^+ and Congored from amniotic fluid in living fetus

Injected Li^+ and Congored started to disappear rapidly from amniotic sac, probably due to increased intraamniotic pressure. Disappearance rate of Congored increased with time as $46.2\pm5.0\%/t$ (Mean$\pm$S.E.) at 30 minutes, $69.4\pm5.5\%/t$ at 60 minutes and $81.6\pm4.7\%$ at 90 minutes (each $p<0.01$). The rate of Li^+ disappearance was higher than that of Congored by $61.8\pm4.37\%/t$ at 30 minutes, $70.4\pm4.05\%/t$ at 60 minutes and $77.3\pm6.03\%/t$ at 90 minutes, respectively (Table 1). A faster rate of disappearance for Li^+ than Congored ($p<0.05$) at 30 minutes suggested the existence of another

Table 1. Disappearance of Li^+ and Congored from amniotic sac with living fetus

	T-time (min)		
	30	60	90
Disappearance rate of Congored (%/t)	46.2± 5.0	69.4± 5.5*	81.6± 4.7***
Disappearance rate of Li^+(%/t)	61.8± 4.37†	70.4± 4.05	77.3± 6.03
Li^+-Congored (%/t)	15.6± 4.65	0.9± 4.65**	−4.9± 8.50**

1 ml of 15 mmole LiCl, 135 mM NaCl and 0.5 gm Congored per litre solution was introduced into amniotic sac.

Disappearance rate of Congored was calculated by $100\times(1\text{-}e^{-kt})$ where disappearance constant (k) was obtained from the plot of logarithm of concentration against time. Disappearance rate of Li^+ was calculated by $100\times$[dose-(volume$\times$t-time conc.)]/dose. Amniotic fluid volume was measured by dilution method using theoretical concentration of Congored at 0-time. Observation numbers were 7 each. Asterisks indicate *$P<0.01$, **$P<0.05$, and ***$P<0.005$ which differ significantly from 30 minutes and †$P<0.05$ from Congored.

Table 2. Disappearance of Li^+ from amniotic sac with umbilical cord ligated fetus

	T-time (min)		
	30	60	90
Disappearance rate of Li^+ (%/t)	27.3± 2.57 (13)	36.7± 3.16 (11)	44.9± 3.23 (11)
Amniotic fluid Volume (ml)	5.6± 0.45 (16)	5.0± 3.79 (11)	4.0± 2.05 (14)*

Amniotic fluid volume was calculated simply by dilution method using t-time concentration of Congored.

Numbers in parenthesis are numbers of amniotic sample.

*significantly different from the corresponding value at 30 min ($P<0.05$).

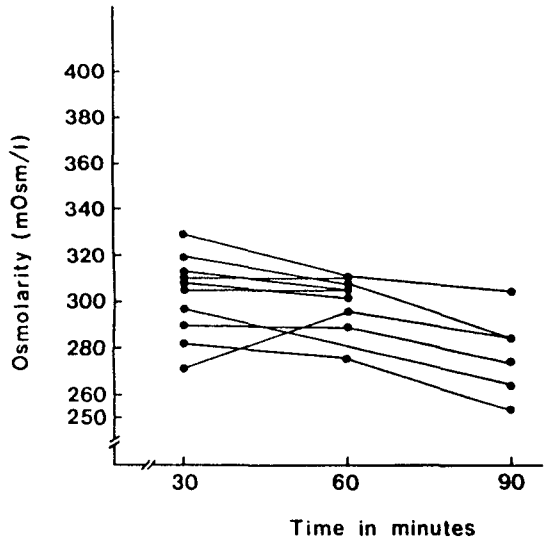

Fig. 1. Time courses of changes in osmolarity of amniotic fluid in living fetus after intraamniotic injection of isosmotic saline. 1 ml of isosmotic saline containing 15 mM LiCl and 0.5 g/L Congored was introduced into amniotic sac. A wide variation of amniotic fluid osmolarity was observed at 30 minutes. The osmolarity tended to decrease with time.

pathway as large as 24.1±6.89% of total transport contributions, other than deglutition of fetus. However, the rates at 60 minutes were similar to one another and reversed by 10.1± 9.88% at 90 minutes. The apparent decrease of the rate for Li^+ was thought to be due to reinflux through fetal urine into the amniotic sac.

Disappearance of Li^+ from amniotic sac in umbilical cord ligated fetus

Li^+ disappearance rates for umbilical cord ligated fetuses were 27.3±2.57% at 30 minutes, 36.7 ± 3.16 % at 60 minutes and 44.9 ± 3.23% at 90 minutes. They were lower compared with those obtained from living fetuses and increased with time, this was not seen in living fetuses. Amniotic fluid volume of expired fetus, estimated by dilution method of Congored, also decreased over time as 5.6±0.45 ml at 30 minutes, 5.0±3.79 ml at 60 minutes and 4.0±2.05 ml at 90 minutes ($p<0.05$) (Table 2).

Alterations in osmolarity of amniotic fluid

Injection of 300 mOsm/L solution into amniotic fluid living fetus (Fig. 1) brought about variable results for amniotic fluid osmolarity, which tended to decrease and recover to 280 mOsm/L as time went by. Changes in osmolarity of amniotic fluid in umbilical cord ligated fetus (Fig. 2) showed wide variations at 30 minutes by the injection of 300 mOsm/L solution. This could be explained by the wide variation of the volumes of amniotic fluids that were influenced by fetal urines of various osmolarity depending on the maturity of fetal kidney. The osmolarity approached the level of normal extracellular fluid during 90 minutes, i.e., low osmolarity increased and high osmolarity decreased.

Changes of Na^+ concentration at 30 minutes

Amniotic Na^+ concentration at 30 minutes varied between 46.4 mEq/L to 157.4 mEq/L as osmolarity changed from 136.0 mOsm/L to 370 mOsm/L, showing a direct correlation (r=

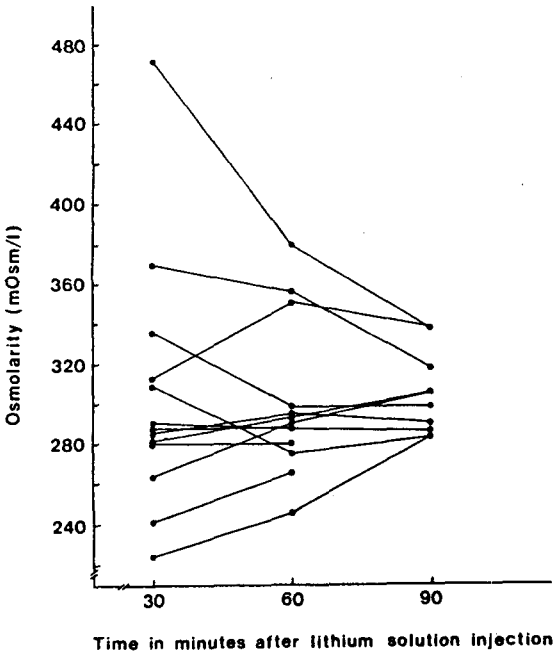

Fig. 2. Time courses of changes in osmolarity of amniotic fluid in expired fetus after intraamniotic injection of isosmotic saline. At 30 minutes, a wide range of osmolarity was observed because of various amniotic fluid volume (see Table 2). The osmolarity narrowed into normal range with time.

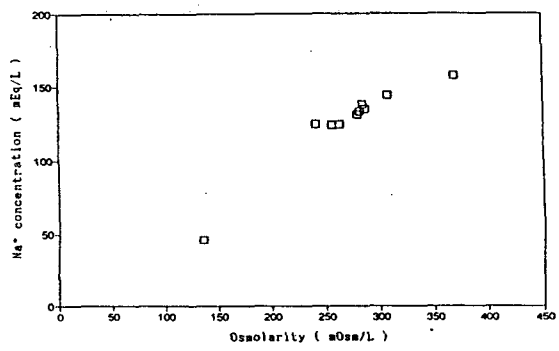

Fig. 3. Relation between the sodium concentration and the osmolarity of amniotic fluid. Amniotic fluid samples were taken 30 minutes after the intraamniotic injection of isosmotic saline. For the linear regression, $r=0.95$, $p<0.001$.

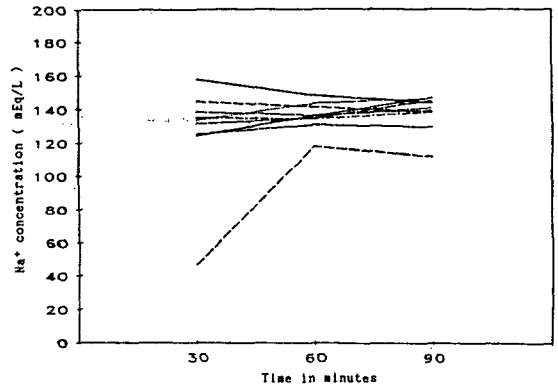

Fig. 4. Time courses of sodium concentration of amniotic fluid in expired fetus after intraamniotic injection of isosmotic saline.

0.95, $p<0.001$) with osmolarity in Fig. 3. Therefore, it was concluded that osmoles of amniotic fluid were composed mainly of sodium ions.

Time course of Na^+ concentration

As seen in Fig. 4, Na^+ concentrations that showed wide degree of variation at 30 minutes had approached toward the level of normal interstitial fluid during 90 minutes; Na^+ concentrations above 144.3 mEq/L at 30 minutes had decreased and those below that had increased. This was similar to the observed changes in osmolarity.

Relation between osmolarity and disappearance rate of Li^+ and Congored

The relation between osmolarity and disappearance rate of Li^+ and Congored at 30 minutes was plotted Fig. 5. The disappearance rate of Li^+ was always higher than that of Congored and the differences between rate of Li^+ and Congored became smaller in the osmolarities above 300 mOsm/L. The relation between osmolarity and disappearance rate of Li^+ in umbilical cord ligated fetus is shown in Fig. 6, where the disappearance rate of Li^+ at 30 minutes, relatively uniform in value below the concentration of 302 mOsm/L, decreased above

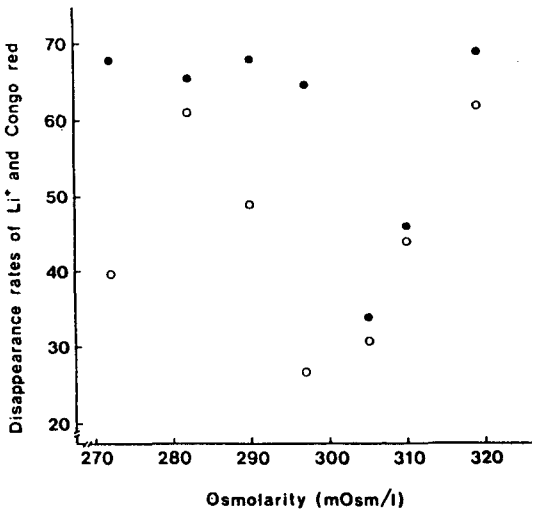

Fig. 5. Disappearance of Li^+ and Congored from amniotic fluid with living fetus as a function of amniotic fluid osmolarity. Diappearance rate of Li (●) within 30 minutes were always higher than those of Congored (○).

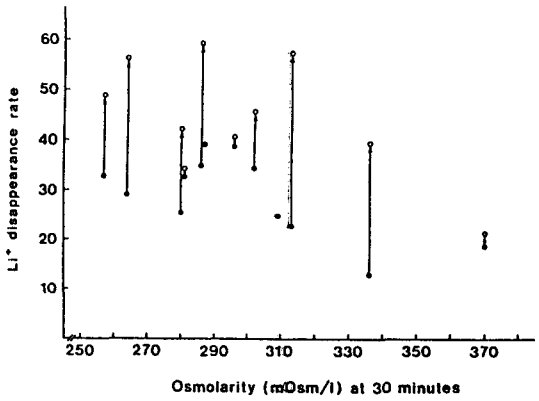

Fig. 6. Disappearance rate of Li^+ from amniotic fluid with expired fetus in relation to its osmolarity. At 30 minutes (●) Li^+ disappearance of amniotic fluid with >308 mOsm/L were lower than those of <302 mOsm/L. At 90 minutes (○) with the changes of amniotic fluid osmolarity toward normal range (see Fig. 2.), differences of rate were also decreased.

the concentration of 308 mOsm/L. This difference, however, was abolished at 90 minutes, when the rate of high osmolarity approached that of low osmolarity. This result was attributed to the decrease of osmolarity with time course.

Time course of amniotic fluid volume

Volumes of amniotic fluid volume measured 30 minutes after injection of 1 ml isoosmotic saline solution showed large individual variation and tended to decrease during the 90 minute time course (see Fig. 7).

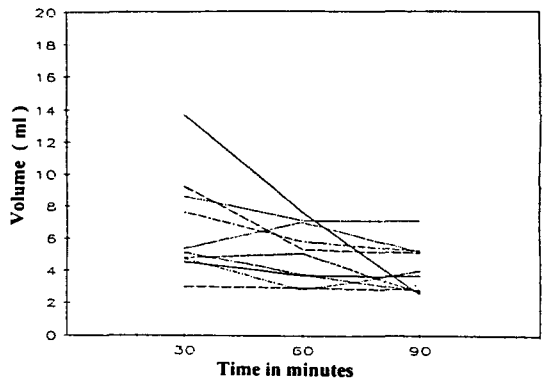

Fig. 7. Changes in the course of time in amniotic fluid volume of the expired fetus after intramniotic injection of isosmotic saline. One ml of isosmotic saline containing 0.5 g/L Congored was introduced into the amniotic sac.

Relation between changes in amniotic fluid osmolarity and volume

As can be seen in Fig. 8, amniotic fluid volume was normalized by the value obtained at 30 minutes, followed by proportionate adjustment. There were small, but significant decrease in amniotic fluid volume after 30 minutes. Amniotic fluid volume had a tendency to decrease after 30 minutes and to further decrease after 60 minutes, although the differences were small. It can be seen that the volumes of amniotic fluid with high osmolarity decreased despite the decrease of osmolarity (Fig. 2).

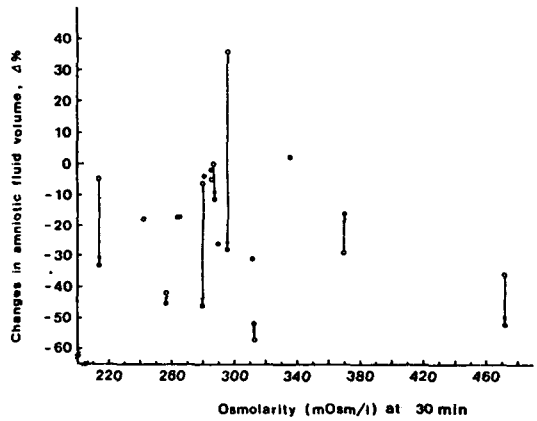

Fig. 8. Changes in volume of amniotic fluid with umbilical cord ligated fetus in relation to its osmolarity. Amniotic fluid volumes were normalized by setting 1 at 30 minutes, with proportionate adjustment of all other volume. Changes of volumes from 30 minutes to 60 minutes (○) and 90 minutes (●) were shown.

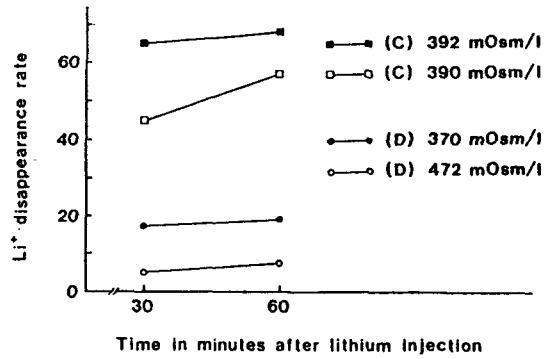

Fig. 9. Li^+ disappearance from hyperosmotic amniotic fluid with living fetus and expired fetus. 1 ml of 15 mmole LiCl plus 185 mmole NaCl per liter solution was introduced into each amniotic sac of living fetus (C) or Li^+ disappearance from amniotic sac with living fetus were within control limit (see Table 1), but with dead fetus, markedly lower rates were observed (see Table 3).

The effect of hyperosmolarity on Li^+ disappearance rate

To confirm the finding that the rate of Li^+ disappearance measured in hyperosmotic amniotic fluid in umbilical cord ligated fetus was low, 400 mOsm/L solution was introduced into the amniotic sac (Fig. 9). In contrast with the rate in the living fetus, which was slightly lower within the range of error (see Table 1) the rate in the ligated fetus became markedly reduced than the rate obtained by administration of isoosmotic solution into the amniotic sac in umbilical cord ligated fetus (Table 2). The rate of Li^+ disappearacne was also measured by administration of mannitol into maternal vessel as illustrated in Fig. 10, where Li^+ disappearacne rate in living fetuses were significantly lower at 30 minutes, though not at 90 minutes, than those of control. Li^+ disappearacne rate in umbilical cord ligated fetuses were within the control limits at all time points (see Table 2).

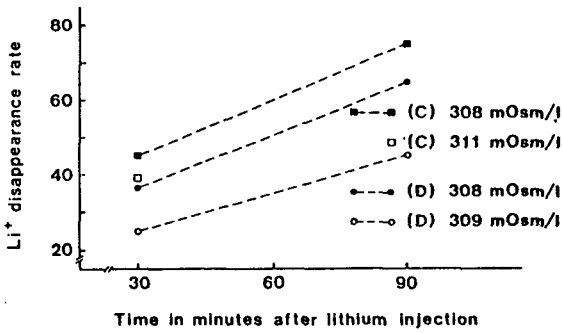

Fig. 10. Effect of mannitol infusion into maternal vein on Li^+ disappearance from amniotic sac with living fetus and expired fetus. Prior to intraamniotic LiCl solution, mannitol (20 ml/kg of a 20% solution) was infused into maternal venous catheter over 10 minutes. In response to maternal mannitol infusion, Li^+ disappearance from amniotic sac in the living fetus (C) were lower only at 30 minutes than those of control (see Table 1) and those from the umbilical cord ligated fetus (D) were identical within control limit (see Table 2).

Table 3. Disappearance rate of Li^+ from hyposmotic and isosmotic amniotic fluid in umbilical cord ligated fetus

Osmolarity at 30 min	T-time (min)		
	30	60	90
Over all	27.3± 2.57 (13)	36.7± 3.16 (11)	44.9± 3.23 (11)
≤302 mOsm/L	33.4± 1.53 (8)*	36.5± 1.55 (6)*	47.2± 3.58 (6)*
≥302 mOm/L	17.6± 2.83 (5)	36.9± 6.70 (5)	42.2± 5.41 (5)

Numbers in parenthesis are sample number of amniotic fluid.

*significantly different from the corresponding value at 30 min ($P<0.05$).

Analysis of Li^+ disappearance rate in umbilical cord ligated fetus

Li^+ disappearance rates in umbilical cord ligated fetuses, showing large variation, were compared with those of the living fetus (see Table 2, 3). In addition, the rates of hyperosmotic amniotic fluids were compared with those of isoosmotic ones. As shown in Table 3 the average Li^+ disappearance rate of isoosmotic amniotic fluid in the expired fetus at 30 minutes was 33.4±1.53%, being half the level obtained in the living fetus, 61.8±4.37% ($p<0.005$). However, at 60 minutes and 90 minutes, when amniotic fluid was becoming isoosmolar (see Fig. 2) Li^+ disappearance rate did not show concentration-dependent differences, therefore, maintaining still lower values than in living fetus group ($p<0.005$).

DISCUSSION

Paul et al. reported in 1956 that half of all water in the amniotic fluid was drained through fetus and the other half through extrafetal pathways. It was Also reported by Lind et al. in 1971 that approximately half the water disappeared via other pathways not through the fetus, since the fetus swallowed only half the volume of fetal urine of which the Na^+ concentration was 75 mEq/L, hypoosmotic compared with 140 mEq/L of Na^+ in amniotic fluid. Therefore, a half to three quarters of total amniotic fluid volume was estimated to be exchanged through fetal swallowing and urination (Friedman et al, 1956) and the rest through the membrane around amniotic fluid (Ross et al, 1983; Brace, 1986). The transport of water through the amniotic membrane is believed to be via diffusion and bulk flow driven by both osmotic and hydrostatic pressure differences (Ross et al, 1983; Lotgering & Wallenburg, 1986). As the appearance rate of maternal D_2O and the disappearance rate of T_2O in amniotic fluid became half the normal level in expired fetus, the transport of water through amniotic membrane was regarded to have a large share in the transport of amniotic fluid (Cittadini et al, 1977; Tomoda et al, 1985).

The injection of mannitol into the maternal vessel increased the maternal plasma osmolarity, accompanied by transport of water into the maternal plasma. This resulted in increased fetal amniotic osmolarity (Ross et al, 1983). However, experimental data on the aspect of pressure difference have not been obtained, possibly due to practical difficulties with the methods used. In comparison with the data that are relatively specific on the transport of amniotic water through amniotic membrane, there is little information on the transport of ions. The constant composition of ions in amniotic fluid was maintained so that after replacement with isotonic mannitol ionic concentrations recovered to normal levels within 6 hours (Canning & Boyd, 1984). The extent of regulation of ion transport by amniotic membrane has yet to be determined, in addition to the previously known fetal urination and swallowing. Extrafetal Na^+ inflow could be presumed to exist, as after ligation of umbilical cord, ^{24}Na ions, previously administered into

maternal plasma, appeared in fetal blood in 10 % degree (Canning & Boyd, 1984). The major extrafetal pathway was presumed to be via the chorio-amniotic membrane. The lithium ion, almost the same size as the hydrated sodium ion, was reported to pass through the Na^+-channel at a far slower rate than the sodium ion (Tosteson, 1981). When administered into maternal plasma, Li^+ showed a lower concentration in the fetus than in the mother, and in amniotic fluid than in fetal plasma (Sung & Kim, 1984). The concentration of Li^+, however, in amniotic fluid rose with time, resulting in a higher concentration in the fetus than in the mother over a long period of Time (Shim & Sung, 1987). Li^+ could flow directly through amniotic membrane as well as through the fetal pathway, and loss of lithium ions from amniotic fluid in the living fetus was greatly influenced by the concentration of lithium ions (Kim et al, 1990). Therefore, the lower rate of loss of Li^+ in spite of its high concentration could be explained in two ways. One explanation was that swallowing amniotic fluid of high Li^+ concentration induced the excretion of Li^+ via urine which added Li^+ into the amniotic fluid. The second explanation was that the amniotic membrane was controlled to restrict the transport of Li^+.

In these experiments the rate of Li^+ disappearance from amniotic fluid of living fetus was higher by far than the rate of Congored disappearance at 30 minutes, suggesting the presence of a Li^+ pathway other than swallowing. The rates became similar at 60 minutes and the rate of Li^+ disappearance decreased at 90 minutes. This suggests that swallowed Li^+ was excreted into the amniotic fluid with an additive effect. Orally administered Li^+ was observed to appear in the urine after 30 minutes (Thomsen & Leyssac, 1986). If fetal Li^+ were excreted through urine in the same way as above, the Li^+ disappearance rate from beginning to 30 minutes might only be regared as real rate. When this rate is compared with the rate obtained in expired fetus where swallowing was impossible, it revealed the presence of pathway other than fetal swallowing, amounting to half of total loss, principally through the amniotic membrane. This agrees with the finding that the rate of disappearance of ampicilline in the expired fetus was about half of that in the living fetus at 30 minutes (Seeds, 1980). The rates measured at 60 minutes and 90 minutes in the expired fetus could also be regarded as real ones, since Li^+ inflow was impossible in this case. So it was clear that injected Li^+ continued to disappear until 90 minutes with a large variation of rates, possibly due to severe changes of amniotic fluid volume and osmolarity. As the osmolarity of amniotic fluid in later stage of pregnancy was reported to be lower by 20 mOsm/L than that of maternal plasma (Lind et al, 1971; Ross et al, 1983) injection of 300 mOsm/L solution into amniotic sac would raise the osmolarity by various degrees according to amniotic fluid volume. Therefore, the osmolarity measured at 30 minutes after injection showed a large variation in the expired fetus in contrast with a small change in the living fetus. Unexpected high osmolarity in some of expired fetuses was interpreted as being due to the high osmolarity of fetal urine, which is usually hypoosmotic, becomes hyperosmotic during short period before full-term (Mellor & Slatter, 1972) and also in stressful conditions to fetus (Seeds, 1980). Therefore, the hyperosmolarity shown in these experiments might be caused either by the stress of umbilical ligation or by the hyperosmotic urine of later stage pregnancy. Amniotic osmolarity as well as Na^+ concentration had decreased to normal levels in the living fetus, whereas it had approached extracellular fluid osmolarity in the expired fetus.

The Li^+ disappearance rate at 30 minutes varied little under normo- or hypoosmotic conditions, but it became low under the condition of hyperosmolarity and its degree of variability became smaller as the amniotic osmolarity approached normal. When NaCl solution of 400 mOsm/L was introduced, the Li^+ disappearance rate varied little within control limits in living fetus, while it decreased in expired fetus. So it was obvious that the increase in

osmolarity, especially the concentration of Na^+, had an inhibitory effect on the rate of Li^+ disappearance. Also the rate showed a great species difference. The time necessary for transporting enough water equivalent to amniotic sac volume was 24 hours in the lamb (Canning & Boyd, 1984), 15 minutes in the rabbit (Paul et al, 1956), 1 hour in the monkey and 3 times longer in man than in the monkey (Friedman et al, 1956).

Congored disappearance rate measured in the living fetus was very high compared with the swallowing rate calculated by disappearance rate of Cr^{51} or RISA (Tomoda et al, 1985). This difference could be attributed to either species variation or to increased amniotic pressure resulting from the injection volume amounting to over 20% of amniotic fluid volume. Though it seemed that the injection of isoosmotic solution would not reduce amniotic fluid volume since it raised osmolarity, amniotic fluid volume decreased with time. It is possible that the reduction of volume was the result of filtration forced by increased pressure developing from injection volume or from fetal death, in which case amniotic fluid volume was reported to decrease by 16% (Paul et al, 1956). This assumption that filtration might contribute to the reduction of volume agreed with the result that fetal urination induced artificially did not produce amniotic fluid volume imbalance or polyhydramnios (Brace, 1986).

The decrease of isoosmotic amniotic fluid volume without osmolarity change was similar to the change in bulk flow caused by increased amniotic pressure, where Li^+ was supposed to be lost by filtration through intercellular spaces of amniotic membrane. When amniotic fluid was hypoosmotic, the filtration seemed to play a major role rather than osmotic bulk flow as the osmolarity recovered to normal level along with decreasing amniotic fluid volume as time went by. Even in case of hyperosmotic amniotic fluid, the filtration along with diffusion seemed to have an influence, though counteracted by osmotic bulk flow, on the composition of amniotic fluid, particularly on osmolarity as amniotic fluid decreased in volume and in osomlarity toward normal level.

The low rate of disappearance of Li^+, similar in size with the Na^+ ion, in hyperosmolar amniotic conditions seemed to be caused by counteraction of osmotic bulk flow upon filtrative bulk flow through the interstitial space of the amniotic membrane (Seeds, 1970). However, at a later time period between 30 minutes to 90 minutes, when the osmolarity and volume of amniotic fluid were reduced to normal control levels, the Li^+ disappearance rate was only 20 %, less than 30% of amniotic fluid volume decreasing rate. The difference between the above two rates suggested Li^+ had diffused out of the amniotic membrane. This is unlike the situation for Na^+ which rarely diffuses since its diffusion rate is thousand times slower than that of water. The increased osmolarity of maternal plasma produced by injecting mannitol had no effct on the Li^+ disappearance rate in the expired fetus. The fact that osmotic bulk flow superimposed on filtrative force failed to influence on the rate further, simiarly to the case in hypoosmotic amniotic fluid suggested a limitation of transport capacity in the amniotic membrane (Kim et al, 1990).

The conclusion which can be drawn from here is that increase of amniotic fluid volume with a resultant increase in pressure was accompanied by filtrative bulk flow and Li^+ loss. In hyperosmotic amniotic fluid, the filtrative bulk flow was partially counteracted by osmotic bulk flow, resulting in decrease of the Li^+ disappearance rate. Therefore, the fact that volume and osmolarity of amniotic fluid were maintained constant, even if fetal swallowing was a half to three quarters of total fetal urine volume and that fetal urine osmolarity was 80 to 140 mOsm/L (Seeds, 1980), suggests that the amniotic membrane allowed the passage of small molecules and water that were driven by collective forces of filtration, osmosis and diffusion. More detailed investigations needed to determine how intraamniotic pressure is increased by amniotic fluid volume challenge and how increased amniotic pressure influences fetal swal-

lowing and amniotic transport.

CONCLUSION

To investigate the effect of osmolarity of the disappearance of Li^+ via the extrafetal pathway during increased amniotic fluid volume the fetuses of 24 pregnant rabbits were used. Fetuses were divided into two main group: 3 living fetuses and 3 expired fetuses made by ligating umbilical cords. All animals were injected into the amniotic sac with 1 ml of isotonic saline solution containing 15 mmole LiCl plus 0.5 gm Congored. The concentrations of Li^+, Congored and osmoles were measured from amniotic fluids at 30, 60 and 90 minutes after the injections of isotonic solutions by sacrificing fetuses in turn. Disappearance rates of Li^+, Congored and amniotic fluid volume were calculated from the above data. The comparison between Li^+ and Congored disappearance rates in living fetus and the time courses of Li^+ disappearance in living and expired fetuses gave the following results.

1. Li^+ disappearance rate of living fetus was higher than Congored disappearance rate at 30 minutes, but was not at 60 and 90 minutes.

2. Disappearance of Li^+ from amniotic fluid in expired fetus was substantial, approaching half the level of that in the living fetus at 30 minutes and continuing over the entire time course.

3. Volume, osmolarity and Na^+ concentration of amniotic fluid in expired fetuses measured after the intraamniotic injection of isoosmotic saline decreased steadily towards the normal extracellular fluid level, though showing wide variations at 30 minutes.

4. Li^+ disappearance rate estimated 30 minutes after the injection of isoosmotic saline in expired fetuses was markedly lower in fetuses of hyperosmotic amniotic fluid than in hypo- or isoosmotic one.

5. Intraamniotic injection of 400 mOsm/L saline solution decreased Li^+ disappearance rate in expired fetuses, but had less effect in living fetuses.

6. Injection of mannitol into maternal vein had no noticeable effect on Li^+ disappearance rate of expired fetus.

From the above results the following conclusions could be made.

1. During volume challenge into amniotic fluid, Li^+ along with water filtrates out via an extrafetal pathway in a sizable amount, however, the rate is reduced when amniotic fluid is hyperosmolar as osmotic bulk flow counteracts filtrative bulk flow.

2. Li^+ and Na^+ continue to diffuse out of amniotic membrane, even after the volume and osmolarity of amniotic fluid have recovered to normal level.

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