Mechanisms of Production and Clearance of Amniotic Fluid

By Fred K. Lotgering and Henk C. S. Wallenburg

The amniotic fluid volume represents a dynamic fetal compartment. Although there is little variation in the size of this compartment at any time during pregnancy, its size and composition changes with gestational age and its turnover rate is high. Inadequate control of amniotic fluid volume may result in oligo- or polyhydramnios, both of which are associated with increased perinatal morbidity and mortality. Therefore, knowledge of the mechanisms involved in amniotic fluid regulation are important from the viewpoint of physiology, as well as obstetrics.

The study of the mechanisms responsible for the production and clearance of amniotic fluid is complicated for several reasons. These include (1) change in size of the amniotic fluid compartment during the course of pregnancy; (2) change in composition of the amniotic fluid with gestational age; (3) variation in fluid volume and, to a lesser extent, in composition between individuals of similar gestational age; (4) presence of several possibly interactive control mechanisms; (5) relative inaccessibility of the amniotic fluid compartment, the fetus, and the placenta and membranes; (6) ethical and legal problems relative to studies in humans; and (7) species differences.

In the following review we have tried to explore what is known and what is not known of the dynamic homeostatic control mechanisms of the amniotic fluid compartment. Our aim has not been to discuss in detail the controversies in the literature, but to put present data into perspective and to add some speculative thoughts for discussion.

Size and Composition

During the second and third trimester of human pregnancy, the volume of total uterine contents, including the fetus, the placenta, and the amniotic fluid compartment, increases almost linearly with gestational age. However, the relative contribution of the three components within the uterus changes markedly during the course of pregnancy. This is demonstrated in Fig 1, which has been composed on the basis of data from the literature. Data points before week 20 are derived from Lind et al., data on total uterine volume from Geirsson et al., and data on fetal and placental weight from Kloosterman. Amniotic fluid volume was calculated by subtracting fetal and placental volumes from the volume of total uterine contents, assuming a specific gravity of 1.0 for all constituents. The calculated amniotic fluid volume is virtually identical to measured values of amniotic fluid volumes, thus supporting the validity of Fig 1.

Amniotic fluid volume shows a steady increase until approximately week 25, and is then followed by a more gradual increase to a peak of about 900 mL at 30 weeks' gestation. Thereafter, amniotic fluid volume decreases, at first slowly, but after 35 weeks more rapidly, and falls sharply after 40 weeks' gestation. Although before week 20 amniotic fluid volume increases almost proportionally to fetal weight, the two variables are not directly related during the second half of gestation. The linear growth of the total of uterine contents represents a rather constant accumulation of water within the uterus. This water may be incorporated into the fetus, the placenta, or the amniotic fluid compartment. One might speculate that after week 30 the fetus may use the water stored in the amniotic fluid compartment to meet the requirements for its rapid growth. This implies fetal control of its amniotic fluid volume, as will be discussed.

The composition of the amniotic fluid in early pregnancy closely resembles that of maternal and fetal serum (Table 1). During the second half of gestation, amniotic fluid osmolality gradually decreases to approximately 92% of the maternal serum value at term, and the concentrations of sodium and chloride decrease by about 5%, while those of urea and creatinine increase by 70% to 250%. Near term, urea and creatinine concentrations in amniotic fluid are higher than those in maternal serum (Table 1), whereas the concentrations of most other solutes are lower. Because fetal...
and maternal serum concentrations are almost identical throughout gestation, similar relationships exist between the amniotic fluid and fetal serum composition.

Most of the work in experimental animals has been performed in pregnant sheep. Because the same trends in amniotic fluid composition have been observed in chronically catheterized pregnant sheep as in humans (Table 1), the sheep may be considered to represent a suitable model for the study of most aspects of human amniotic fluid dynamics.

PATHWAYS OF EXCHANGE

The amniotic cavity is a fetal compartment surrounded by fetal structures (Fig 2). Although the amniotic fluid is ultimately derived from the mother, all its components must pass fetal structures (chorioamnion or fetal-placental circulation) before reaching the amniotic cavity. This offers the possibility of fetal control over this compartment, either passively by its membrane characteristics, or actively by fetal homeostatic control mechanisms, within limits set by the mother. The fetal urinary tract and, to a lesser extent, the respiratory tract are known to contribute to the production of amniotic fluid, whereas swallowing and probably transport across the chorioamnion are major sites of amniotic fluid clearance. In addition, some quantitatively less-important exchange may occur across the umbilical cord and the fetal skin, as well as other sites.

Urinary Tract

It has been firmly established that fetal urinary output is a major factor in amniotic fluid production and becomes progressively more important with advancing gestational age. Urine has been demonstrated to be present in the fetal bladder from 11 weeks onwards. Urine production in the human fetus increases in a linear fashion from 3.5 mL/h at 25 weeks' gestation to 26 mL/h at 39 weeks, and falls sharply thereafter. Because the relationship between fetal weight and gestational age is also virtually linear between 25 and 39 weeks, urine production in the human increases in proportion to fetal weight from ~110 mL/kg·24 h at 25 weeks, to ~190 mL/kg·24 h at 39 weeks' gestation.

With the increase in fetal urinary volume during the course of gestation, progressively more oncortically active substances are added to the amniotic fluid (Table 2). Nonetheless, amniotic fluid volume at 38 weeks is similar to that at 20 weeks, and osmolality is less (Fig 3). Consequently, progressively more water, electrolytes, and solutes must be removed from the amniotic fluid compartment with advancing gestation.

Fetal urinary output is the result of fetal renal function. The glomerular filtration rate near term has been calculated to average 34 mL/min·1.73 m² (range 16 to 58 mL/min·1.73 m²), which is about

<table>
<thead>
<tr>
<th>Duration of Pregnancy (weeks)</th>
<th>Osmolality mosm/kg</th>
<th>%</th>
<th>Sodium mmol/L</th>
<th>%</th>
<th>Urea mmol/L</th>
<th>%</th>
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<tbody>
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<td>91</td>
<td>124</td>
<td>91</td>
<td>5.3</td>
<td>168</td>
</tr>
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</table>

*Given in absolute figures and related to maternal serum concentration (%) at 20, 30, and 40 weeks' gestation. Adapted from Lind.8
one fourth of the normal adult value. Tubular reabsorption in the same study averaged 90%, with variation between 76% and 95%. This suggests that the human fetal kidney is actively functioning. In addition, studies in sheep have demonstrated that the fetal kidney in the last quarter of gestation responds to vasopressin by free water reabsorption and increased urinary osmolality, although to a lesser extent than in adults.\textsuperscript{10,11} This is accompanied by increased osmolality and electrolyte concentrations of the amniotic fluid.\textsuperscript{12} Changes in fetal blood volume also affect urine output. A 20% reduction in fetal blood volume in sheep, associated with a reduction in cardiac output and renal blood flow, was found to reduce fetal glomerular filtration rate by 19% and urinary output by 76%, while the urine-to-plasma osmolality ratio increased.\textsuperscript{13} In the same study, expansion of fetal blood volume was observed to increase cardiac output and renal blood flow, as well as to enhance urine output. These data demonstrate that the fetal kidney effectively responds to stimuli involved in maintaining fetal blood volume, at least in late gestation.

**Respiratory Tract**

The fetal lung represents a site of significant fluid production. During the last 30 days of gestation in sheep, the amount of lung fluid produced increases in a linear fashion from about 30 mL/kg·24 h to 50 mL/kg·24 h.\textsuperscript{14} The amount of fluid produced by the lung is probably subject to fetal control, as vasopressin has been demonstrated to reduce markedly the production of tracheal fluid in fetal sheep, without significantly affecting the osmolality or electrolyte concentrations.\textsuperscript{15} Nonetheless, this may not directly affect amniotic fluid volume regulation, because a variable amount of tracheal fluid will be swallowed before it reaches the amniotic fluid compartment. However, when swallowing does not take place, as in esophageal atresia, tracheal fluid production can be expected to contribute significantly to the production of amniotic fluid volume. Although little is known about the tracheal fluid production in human fetuses, the appearance of surface-active phospholipid during the third trimester of human pregnancy demonstrates that some of the tracheal fluid reaches the amniotic cavity.

<table>
<thead>
<tr>
<th>Duration of Pregnancy (weeks)</th>
<th>Amniotic Fluid Volume (mL)</th>
<th>Fetal Weight (kg)</th>
<th>Urine Concentration</th>
<th>Urinary Production</th>
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<td>(mosmol/kg)</td>
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<td>Sodium (mmol/L)</td>
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<td>Urea (mmol/L)</td>
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<td></td>
<td></td>
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<td>1.7</td>
<td>10.2</td>
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Adapted from Wallenburg.\textsuperscript{27}
Absorption of amniotic fluid by the fetal lungs has been suggested as an important mechanism for amniotic fluid clearance because polyhydramnios may develop in case of aplasia of the trachea. However, absorption of amniotic fluid by the lungs has been demonstrated not to occur to any significant extent in the normal human fetus, as contrast medium is not present in the fetal lung after amniography and only minimal amounts of radioactivity are found in the fetal lungs after injection of radioactive colloidal gold into the amniotic sac.

Gastrointestinal Tract

Swallowing represents an important mechanism of clearance of amniotic fluid. Amniographic studies have demonstrated radiopaque dye in the fetal intestines within 20 minutes after injection of the dye into the amniotic cavity. Quantitatively, this mechanism becomes increasingly important with advancing gestational age, as demonstrated in a small number of human fetuses by the disappearance of chromium-labeled red cells from the amniotic cavity after injection. In these studies fetal swallowing of amniotic fluid increased from 7 mL/24 h at 16 weeks to about 500 mL/24 h at term, or from about 50 mL/kg-24 h to 150 mL/kg-24 h. Near term, the amount of fluid swallowed per day almost equals the total amniotic fluid volume. With the use of a similar technique, the amount of amniotic fluid removed by swallowing was determined in sheep and appeared to be approximately 375 mL/kg-24 h, which is markedly higher than the above-cited human values. Whether the lower swallowing rate observed in human fetuses represents an artefact resulting from the small number of observations, or whether this represents a true species difference remains to be determined. However, it should be realized that with the use of this method, only the amount of swallowed amniotic fluid can be measured, and that tracheal fluid removed by swallowing before mixing with amniotic fluid remains unnoticed. Therefore, the total volume of swallowed fluid may be approximately 30% higher than suggested by the above figures.

Despite the large volume swallowed by the fetus each day, experimental jejunoileal atresia or ligation of the esophagus does not result in any significant alteration in amniotic fluid volume or composition. Although this might suggest that swallowing is not an important factor in amniotic fluid homeostasis, an alternative explanation could be that the reduced intake of fluid is fully compensated by the fetus, eg, by reduced production of urine and/or tracheal fluid, or by some other mechanism.

Swallowing occurs in a variable number of episodes per day, each of which may last from one to several minutes. As early as 1937 it was suggested that the fetus modifies its swallowing behavior in response to chemical stimuli. This was contradicted by later studies in fetal sheep that failed to demonstrate a significant effect of glucose or a bitter-tasting chemical on the amount of fluid swallowed per day. Although taste may not significantly affect the amount of fluid swallowed per day, one might speculate that thirst, decreased circulatory blood volume, or increased osmolality of the fetal blood might stimulate fetal swallowing and, consequently, affect amniotic fluid regulation. One might speculate that in cases of anencephaly, polyhydramnios could develop from in-
adequate swallowing as a result of a defect in any of these steps in the absence of adequate compensation.

Placenta and Membranes

The implications of the anatomical structures of amnion, chorion, and placenta for the transport of water, electrolytes, and other solutes from and to the amniotic fluid have been discussed in some detail in previous reviews.\textsuperscript{27-29} Many of the amnion cells are separated by intercellular channels, which allow for bulk flow across this membrane in vitro. However, because the vascularity of maternal tissue in proximity to the chorion laeve is sparse, the net transfer of water across the reflexed membranes can only be small.\textsuperscript{29} This is markedly different at the site of the placenta, where the chorioamnion is in close contact with the maternal blood, and significant exchange may take place.\textsuperscript{29}

Two different types of water exchange have been demonstrated to take place across the amnion and chorion: fast nondiffusional bulk flow through intercellular channels, and slower diffusional flow. Both mechanisms of exchange are governed by hydrostatic and osmotic gradients. In contrast to their high permeability to water, the membranes are impermeable to many compounds in excess of 1,000 mol wt,\textsuperscript{29} while smaller solutes, such as sodium, glucose, urea, etc, cross by simple diffusion.\textsuperscript{30} Consequently, the overall membrane characteristics are those of a partially semipermeable membrane. Because of the inaccessibility of the fetal membranes, the study of water exchange across the membranes in vivo is difficult and restricted to radioactive tracer studies. Although such studies are elegant in design, they may be subject to considerable error. The most commonly quoted study in humans resulted in the following calculated net transfer rates: fetus to mother 25 mL/h, amniotic fluid to fetus 16 mL/h, and mother to amniotic fluid 18 mL/h.\textsuperscript{31} These values suggest that the fetus is in a negative water balance, an assumption that is unlikely to be correct in the steady state.

In vivo, net water transfer occurs as the result of the net imbalance between the hydraulic and osmotic forces across the membranes. The inability relative to hydraulic permeability of a compound to cross the membranes in response to an osmotic gradient is represented by the reflection coefficient ($s$); the reflection coefficient for albumin is 1.0, whereas that for a small solute like urea is 0.02 for amnion, and 0.01 for chorion.\textsuperscript{29} The effective osmotic pressure difference across a membrane of a solute can be described by adding $s$ to the van 't Hoff equation:

$$\Delta \Pi_i = s_i \Delta mRT,$$

in which $\Delta \Pi_i$ is the effective osmotic force across the membrane for solute $i$, $s_i$ is the reflection coefficient, $\Delta m$ is the concentration difference, $R$ is the universal gas constant, and $T$ is the absolute temperature. The effective total osmotic force is the mathematical sum of the effective osmotic forces of all solutes present on either side of the membrane. It has been argued that, as a result of marked differences between reflection coefficients for different solutes, the effective total osmotic force across the membranes may be quite different from the gradient in measured overall osmolality, and may even result in water transfer against this gradient.\textsuperscript{29} However, with the exception of urea and creatinine, which have low reflection coefficients, the steady state concentration of most solutes is lower in amniotic fluid than in maternal serum throughout normal pregnancy. Consequently, the sum of effective osmotic forces will favor the transport of water from the amniotic fluid compartment towards the maternal circulation throughout gestation. If one would accept osmolality as a crude estimate of total effective osmotic force, the amniotic-to-maternal osmolality difference of 6 mosm/L at 20 weeks and 24 mosm/L at 38 weeks' gestation would indicate that the net amount of water leaving the amniotic fluid compartment across the amnionchorion as a result of osmotic force at 38 weeks would be four times as high as that at 20 weeks. Although the permeability of the amnion and chorion to water is unaffected by gestation,\textsuperscript{32} in vitro studies suggest that prolactin may reduce amniotic permeability to water,\textsuperscript{33} and animal studies suggest that this may affect amniotic fluid volume.\textsuperscript{34} If prolactin would indeed affect the reflection coefficients of the membranes, this could affect estimated effective total osmotic force in an unpredictable manner and might explain the fourfold reduction in disappearance rate of labeled urea from the amniotic fluid between midgestation and term in sheep without a similar change in the disappearance rate of water.\textsuperscript{35} In addition, transfer of one solute may be facilitated by coupling to another solute or to wa-
ter, mechanisms which have not been studied in relation to transport across the amniotic and chorionic membranes. Consequently, the net flux of water from the amniotic cavity to the mother across the amnion and chorion cannot be assessed accurately at the present time.

Under unsteady conditions osmotic forces may be markedly different from those in the steady state. A maternal osmotic challenge results in bulk flow of amniotic fluid water to the mother and in increased amniotic fluid osmolality. Since the fetal kidney is functionally capable of affecting the urine-to-plasma osmolality ratio, amniotic fluid osmolality and water flux across the amnion and chorion may be affected by a variety of fetal conditions, eg, blood volume changes, large glucose variations, renal function disorders, etc. Osmotic challenge from fetal sources, eg, trachea or bowel, are also likely to affect water flux across the placental and reflexed membranes.

Apart from osmotic forces, other mechanisms might affect the transfer of water and solutes across the amniotic and chorionic membranes. If a hydraulic gradient would exist across the membranes, bulk flow of water would result. With intact membranes, such a gradient cannot exist between the intrauterine compartments because water is incompressible. However, one might speculate that in the presence of ruptured membranes maternal intervillous space pressure could slightly, but permanently, exceed amniotic fluid pressure. This would result in bulk flow of water towards the amniotic fluid compartment proportional to the hydraulic pressure difference. Hydraulic forces have not been studied experimentally in relation to amniotic fluid regulation.

Amniotic epithelial cells contain an extensive rough endoplasmatic reticulum indicating protein synthesis, especially in early pregnancy, while at term, lipids are a prominent feature. This suggests synthesis and/or secretion of proteins and lipids, some that (such as prolactin?) may act to modulate membrane characteristics, while others may act through their osmotic force. Although the importance of their function is poorly understood at present, they may help to explain the observation that amniotic fluid is present in early pregnancy, even in the absence of a fetus. At present there is no convincing evidence of the existence of active energy-requiring transport of any compound across the membranes.

As a result of the complexity of the quantitative and qualitative aspects of transport between amniotic fluid and maternal blood, the net flux of water and solutes across the amniotic and chorionic membranes in vivo cannot be accurately assessed at the present time.

Other Exchange Sites

The fetal skin in early pregnancy consists of only a few cell layers interposed between a basal and a superficial cell layer. The superficial cell layer, or periderm, gradually disappears by 17 to 20 weeks. Until then, these cells contain intracellular structures suggestive of functional activity. In vitro experiments have shown permeability to water and sodium in early gestation, and in vivo experiments have demonstrated transport of urea and electrolytes in early midpregnancy. After keratinization, which is complete at about 25 weeks' gestation, the fetal skin must be considered to be impermeable.

The umbilical cord has been considered a possible site of transfer of water between the amniotic fluid and the fetal circulation. Both in vitro and in vivo experiments have demonstrated bidirectional water flux across the amniotic cord. However, it has been argued that the umbilical cord is a totally ineffective site of exchange and a physiologically important net transfer of water has not been satisfactorily demonstrated.

Other potential exchange sites, which might include the oral and nasal mucosa, are probably also quantitative of little significance and have not been studied.

CONTROL OF REGULATION

The one objective of all living organisms is the preservation of a constant environment. Intricate homeostatic mechanisms preserve the internal milieu of mammals, thereby limiting their dependence on a constant external environment. During pregnancy, the mother provides a rather constant external environment to the products of gestation. Although this may be adequate in early pregnancy, with advancing gestation, the fetus becomes more and more dependent on its own homeostatic mechanisms as a result of its size, within the limits set by maternal supply. During the second half of human gestation, the products of gestation, which include the fetus, the placenta, and the amniotic fluid, accumulate water at a constant rate of about
25 mL/d (Fig 1). The reasons for this rather constant accumulation rate are poorly understood. However, clinical observations suggest that supply, demand, and transfer may all affect the rate of accumulation. On the other hand, maternal vascular disease and uterine anomalies are associated with reduced growth, which is suggestive of reduced supply; the small size of the uterus in case of genetically small infants suggests limited demands; and reduced growth after placenta infarction suggests a transfer problem. On the other hand, the rate of accumulation is increased in multiple pregnancy, diabetic infants, fetal hydrops, and in cases of polyhydramnios. Most likely, this results from increased fetal demands mediated by facilitated transfer and liberal maternal supply.

Given the concept of limitation to volume accumulation at any time during gestation, redistribution between the fetal compartments in humans resembles the growth process as it takes place in a bird’s egg. When at one point in gestation the rate of uterine fluid accumulation is reduced for reasons of either supply, demand, or transport, redistribution will allow the fetus to continue to grow at the cost of the amniotic fluid compartment. This may be the case in such clinical conditions as postdate pregnancy and “uteroplacental insufficiency,” both of which are associated not only with a reduction in fetal growth rate, but also with a more pronounced decrease in amniotic fluid volume.

Most intensive exchange between mother and fetus takes place at the placenta, where both circulations are in close contact. Permeability to water at this site of exchange is virtually unlimited, and water flux is governed by the hydrostatic and osmotic forces on both sides of the placenta. Apart from the daily accumulation of water, the amount of water entering the fetus must be identical to the volume leaving the fetus. As demonstrated in Fig 4, the only sites at which water is known to leave the fetus in significant net amounts are the urinary tract (~1,000 mL/d) and the respiratory tract (~250 mL/d). Through swallowing ~250 mL/d of tracheal fluid and ~750 mL/d of amniotic fluid, or a total of ~1,000 mL/d reenters the fetus. The remaining ~250 mL/d of excreted fetal water must then be removed from the amniotic fluid compartment through some other pathway, most likely through diffusion across amnion and chorion to the intervillous space, and the same amount must reenter the fetal circulation to maintain homeostasis.

The quantitative importance of this last pathway is determined by the difference between fetal excreted water (~1,250 mL/d in urine and tracheal fluid, and absorbed water ~1,000 mL/d through swallowing). The values used to calculate this difference were derived from various sources in the literature and may thus be subject to considerable error. Therefore, at present one cannot exclude the possibility that the pathway amnion-chorion-intervillous space-fetal circulation in normal term pregnancy is either virtually nonexistent or quantitatively more important than estimated above. Studies are presently underway to further quantify the various pathways simultaneously within the same fetus. The same mechanisms involved in maintaining a constant fetal internal milieu seem to be involved in maintaining a relative constant volume and composition of the amniotic fluid com-
partment. Through control of BP and blood volume, the fetus may affect umbilical uptake and delivery of water and solutes, renal function, tracheal effusate production, and, in association with the CNS, thirst and gastrointestinal uptake of water. The central role of fetal blood volume regulation in the regulation of amniotic fluid volume will be discussed in greater detail by R.A. Brace, elsewhere in this issue of Seminars.

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The relative constancy of the amniotic fluid compartment at any time during normal gestation, in the presence of a high turnover rate and a large number of potential pathways of exchange, demonstrates the remarkable effectiveness of the coordination. Probably oligo- and polyhydramnios represent the result of inadequate control of coordination, rather than a disturbance in a single pathway of amniotic fluid production or clearance.