THE PHYSICAL PROPERTIES AND CHEMICAL COMPOSITION OF HUMAN AMNIOTIC FLUID.

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An inquiry into the biological meaning of the human amniotic fluid demands, first of all, a sufficient knowledge of its physical properties and chemical composition, information which many investigators have attempted to gain. Prochownick (1) studied the sodium chloride, solid substances, and urea in the amniotic fluid; Schroeder (2) determined the quantities of solid substances, ash, and albumins; Farkas and Seipiades (3) inquired into its physical properties and into the quantity of sodium chloride contained in it; Jacqué (4) and Grünbaum (5) measured its freezing point. These reports, however, are defective either in the methods used, or in the statement of their examinations; accordingly, in many cases, the results are highly problematical. Döderlein (6) and Jacqué (4) performed some fairly exact analyses of amniotic fluid of mammalia, but their results cannot be applied directly to human amniotic fluid.

Physical Properties of Human Amniotic Fluid.

A study of the literature of the amniotic fluid may be said to establish the following facts: the fluid has a specific gravity varying from 1.002 to 1.028; a freezing point which varies from $\Delta = -0.45$ to $-0.5^\circ$; it is always hypotonic against the blood of the maternal body and the fetus. On the basis of these facts, most investigators have concluded that the amniotic fluid is not a simple transudate, but a mixture of fetal urine with maternal transudate.

Experimental Material.

The urine of women in labor was removed through a Nelaton's catheter after disinfection of the vulva; a sterile dry cylindrical speculum was in-
Amniotic Fluid

Introdued deep into the vagina, and the os uteri and its circumference were wiped with sterile gauze, thereby cleansing it entirely from blood, mucous particles, etc. The fetal membranes were pierced with a sharp instrument, and the amniotic fluid flowing out from the inside of the womb was collected in a clean glass flask, and preserved with a small quantity of toluene in an ice box. Thus obtained, the amniotic fluid is almost pure, since it has little chance to become impure, as it flows along the sides of the speculum, without touching the mucous membrane of the vagina or the skin of the vulva.

The amniotic fluid is slimy, yellowish white or pale yellow, and cloudy like soap water (rarely clear); it almost always contains much mucous flocks and has a peculiar odor. Because of its sliminess, the fluid is first filtered through fourfold gauze and later with suction. Thus prepared, the slightly cloudy liquor was used for the following experiments.

Specific Gravity.

With a 5 or 10 cc. pyknometer, the specific gravity of the amniotic fluid of women at the end of pregnancy was determined in twenty-three cases, and was found to average 1.0078. In six cases, the liquor was yellowish green, mixed with meconium, with a specific gravity varying from 1.0081 to 1.0136, averaging 1.0106; in the remaining seventeen cases, the liquor was yellowish white or yellow, and the specific gravity varied from 1.0046 to 1.0099, averaging 1.0069. Furthermore, in one case in the 5th month of pregnancy, in which artificial abortion was performed because of tuberculosis, the specific gravity of the fluid was 1.008, and in another case at the beginning of the 6th month, it was 1.0087. The admixture of meconium always causes an increase of the specific gravity. In one instance, in the first half of pregnancy, no evident difference was found.

The relation between the specific gravity and the freezing point is shown in Table I.

Freezing Point and Osmotic Pressure.

By use of Beckmann's cryoscopic apparatus, the freezing point of the amniotic fluid of twenty-two women at the end of pregnancy was determined. The value of Δ was found to vary from 0.46 to 0.565°, with an average of 0.504°. In sixteen cases, the amniotic fluid was yellowish or yellowish white, with an average freezing point of 0.504°; in the other six cases, the liquor was yellowish green, mixed with meconium, and had an average freez-
### TABLE I.

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According to these results, it seems that the admixture of meconium has no marked influence upon the freezing point. The amniotic fluid of a pregnant woman at the beginning of the 6th month showed the freezing point \( \Delta \) to be 0.54°.
From the formulas $P = 0.082\, CT$ (in which $P$ = osmotic pressure, $C$ = osmotic concentration, and $T$ = absolute temperature) and $C = \frac{\Delta}{E}$ (in which $\Delta$ = the lowering of freezing point, and $E$ = a constant), the osmotic pressure of the liquor at 0° was calculated (Table I). The values for the sixteen cases of pure amniotic fluid varied from 5.536 to 6.80 atmospheres, with an average of 6.072, while those of the six cases in which the fluid was mixed with meconium varied from 5.656 to 6.80, with an average of 6.081 atmospheres.

**Specific Electrical Conductivity.**

The resistance of twenty-three samples of amniotic fluid was measured by the usual method, and the specific electrical conductivity calculated from the formula $U = \frac{C}{W}$ ($W$ = resistance, $C$ = resistance capacity). The resistance capacity was determined by using a 0.1 N KCl solution. In seventeen cases, the amniotic fluid was yellowish and the specific electrical conductivity varied from $119.06 \times 10^{-4}$ to $134.00 \times 10^{-4}$, with an average of $127.15 \times 10^{-4}$. In six cases, the fluid was yellowish green, mixed with meconium, and the specific electrical conductivity varied from $120.62 \times 10^{-4}$ to $132.84 \times 10^{-4}$, with an average of $126.90 \times 10^{-4}$. The admixture of meconium seems to have no marked influence on the electrical conductivity.

**Hydrogen Ion Concentration.**

According to Hasselbalch's (7) method, a gas electrode was made with amniotic fluid, and connected with a calomel electrode (0.3377 volt), an accumulator whose electromotive force was previously determined in comparison with a cadmium standard cell, a resistance bridge, and a capillary electrometer; the electromotive force of the fluid was determined, and from this the H ion concentration was calculated. The results with the H ion concentration are shown in Table II.

In sixteen cases, the amniotic fluid was pure, and the H ion concentration varied from $0.2266 \times 10^{-8}$ to $0.2648 \times 10^{-7}$, with an average of $0.1282 \times 10^{-7}$. In five cases, the liquor was yel-
TABLE II.

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<td>0.8338 X 10^-8</td>
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<td>Still-born, mixed with meconium.</td>
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lowish green, mixed with meconium, and the H ion concentration varied from 0.6131 X 10^-8 to 0.1453 X 10^-7, with an average of 0.8903 X 10^-8. It is evident from these figures that the reaction of the amniotic fluid is slightly alkaline. In addition, the reaction was tested with litmus paper; in thirteen cases, the reaction was weakly alkaline, in nine cases it was neutral; in no case was the reaction acid.
Amniotic Fluid

Optical Activity.

The albumin was coagulated by adding a small quantity of sodium chloride, making the solution slightly acid with 1 per cent acetic acid, and heated on a water bath. After filtering, only one of the twenty-two samples examined showed any optical activity (−0.01°).

Summary.—(1) The specific gravity of pure amniotic fluid averages 1.0069 and never rises higher than 1.010. (2) The freezing point averages 0.504°; the difference between the greatest and the smallest amounts to nearly 0.1°. The osmotic pressure averages 6.072 atmospheres. (3) The specific electrical conductivity averages $127.15 \times 10^{-4}$, and corresponds nearly to that of a 0.1 N solution of KCl. (4) The average H ion concentration is $0.1282 \times 10^{-7}$; that is, slightly alkaline. (5) The reaction with litmus paper is either slightly alkaline or neutral. (6) It is nearly optically inactive.

Inorganic Components of Human Amniotic Fluid.

Farkas and Scipiades (3) found 0.444 to 0.58 per cent (average 0.507 per cent) sodium chloride in the amniotic fluid of five pregnant women. Jacqué (4) analyzed the amniotic fluid of eighteen sheep fetuses with a length of 2 to 49 cm. and found: insoluble ash 0.017 per cent, soluble ash, 0.82 per cent, sodium chloride 0.64 per cent, and total ash 0.84 per cent. He also analyzed the fluid of two swine and found: insoluble ash 0.024 to 0.030 per cent, soluble ash 0.74 to 0.76 per cent, sodium chloride 0.53 to 0.55 per cent, and total ash 0.77 to 0.78 per cent. Döderlein (6) performed exact analyses of the amniotic fluid of fifteen cows and found: Cl 0.358 per cent, NaCl 0.586 per cent, Na₂O 0.367 per cent, K₂O, 0.060 per cent, Ca 0.014 per cent, Mg 0.0038 per cent. Nauta (8) found 0.36 per cent chlorine, 1.44 per cent solid substances, and 0.59 per cent ash in the amniotic fluid of cows.


The filtered amniotic fluid was centrifuged for 30 minutes, freed from sediment, and filtered with suction. A known quantity of this filtrate, which in most cases was still a little cloudy, was evaporated on a water bath, and dried to constant weight.
at 110°C., giving the solid substance. This was then ashed and separated into soluble and insoluble ash. The sum of the two is total ash; the organic substances and the water were obtained by difference. The results of the analyses are shown in Tables III to VI.

### TABLE III

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<th>Low</th>
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<td>99.76</td>
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<td>Solid substances (26 cases)</td>
<td>1.24</td>
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<td>Soluble ash (20 cases)</td>
<td>0.77</td>
<td>0.85</td>
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<td>Insoluble ash (20 cases)</td>
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<td>Total ash (20 cases)</td>
<td>0.81</td>
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<td>Organic substances (20 cases)</td>
<td>0.45</td>
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### TABLE IV

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<td>9</td>
<td>20</td>
<td>1.77</td>
<td>99.13</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>11</td>
<td>20</td>
<td>1.95</td>
<td>99.04</td>
<td>&quot; &quot; &quot;</td>
</tr>
<tr>
<td>Average.</td>
<td></td>
<td>1.82</td>
<td>99.30</td>
<td></td>
</tr>
</tbody>
</table>

* Excluded from the average.
### TABLE V.

<table>
<thead>
<tr>
<th>No. of amniotic fluid</th>
<th>Gm. per 100 cc. fluid.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>16 25</td>
<td>1.19 0.75 0.04 0.79 0.40 99.54</td>
<td>Mixed with a little meconium.</td>
</tr>
<tr>
<td>17 20</td>
<td>1.32 0.72 0.04 0.76 0.56 99.76</td>
<td></td>
</tr>
<tr>
<td>18 20</td>
<td>1.02 0.72 0.04 0.76 0.27 99.63</td>
<td></td>
</tr>
<tr>
<td>19 20</td>
<td>1.53 0.77 0.04 0.81 0.72 99.29</td>
<td></td>
</tr>
<tr>
<td>20 20</td>
<td>1.25 0.78 0.04 0.82 0.43 99.50</td>
<td></td>
</tr>
<tr>
<td>22 15</td>
<td>1.23 0.80 0.04 0.84 0.39 99.43</td>
<td></td>
</tr>
<tr>
<td>23 25</td>
<td>1.15 0.74 0.04 0.78 0.76 99.49</td>
<td></td>
</tr>
<tr>
<td>24 20</td>
<td>1.35 0.75 0.04 0.78 0.57 99.39</td>
<td></td>
</tr>
<tr>
<td>27 20</td>
<td>1.13 0.72 0.04 0.76 0.37 99.33</td>
<td></td>
</tr>
<tr>
<td>29 15</td>
<td>1.41 0.81 0.04 0.85 0.55</td>
<td></td>
</tr>
<tr>
<td>Average.</td>
<td>1.26 0.76 0.04 0.80 0.46 99.48</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE VI.

<table>
<thead>
<tr>
<th>No. of amniotic fluid</th>
<th>Gm. per 100 cc. fluid.</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 20</td>
<td>1.27 0.76 0.04 0.80 0.46</td>
<td>Mixed with a little meconium.</td>
</tr>
<tr>
<td>35 20</td>
<td>1.24 0.79 0.03 0.82 0.42</td>
<td></td>
</tr>
<tr>
<td>37 20</td>
<td>1.34 0.73 0.05 0.78 0.56</td>
<td></td>
</tr>
<tr>
<td>39 20</td>
<td>1.16 0.78 0.05 0.83 0.33</td>
<td></td>
</tr>
<tr>
<td>40 20</td>
<td>1.07 0.72 0.04 0.76 0.31</td>
<td></td>
</tr>
<tr>
<td>41 20</td>
<td>1.42 0.84 0.04 0.88 0.54</td>
<td></td>
</tr>
<tr>
<td>44 20</td>
<td>1.14 0.79 0.03 0.82 0.32</td>
<td></td>
</tr>
<tr>
<td>47 20</td>
<td>1.50 0.76 0.04 0.79 0.70</td>
<td></td>
</tr>
<tr>
<td>49 20</td>
<td>1.22 0.80 0.04 0.84 0.38</td>
<td></td>
</tr>
<tr>
<td>50 20</td>
<td>1.26 0.85 0.04 0.90 0.36</td>
<td></td>
</tr>
<tr>
<td>Average.</td>
<td>1.26 0.78 0.04 0.82 0.44</td>
<td></td>
</tr>
</tbody>
</table>
D. Uyeno

Quantitative Determination of Inorganic Components.

The soluble ash was dissolved in water; chlorine, sulfuric acid, phosphoric acid, potassium, sodium, calcium, and magnesium were determined. The insoluble ash was dissolved in hydrochloric acid, then diluted with water, and used for the determinations of sulfuric acid, phosphoric acid, calcium, and magnesium.

Chlorine was precipitated and weighed as silver chloride. Sulfuric acid was precipitated as barium sulfate. Phosphoric acid was determined in the aqueous solution or hydrochloric acid solution by Neumann's (9) method.

Potassium and Sodium.—Phosphoric acid and sulfuric acid were removed as insoluble barium salts; ammonium carbonate solution and ammonia were added to the filtrate to precipitate the excess barium; the filtrate was evaporated after adding a drop of hydrochloric acid; the ammonium carbonate and ammonium chloride were removed by heating, and the residue was weighed as total alkali chlorides. The quantity of potassium in these alkali chlorides was determined as potassium platinic chloride, and the quantity of sodium determined by difference.

Calcium.—The aqueous or hydrochloric acid solution was treated with liquid ammonia till it reacted strongly alkaline; it was then made acid with acetic acid, and the precipitate of iron salts filtered. The filtrate was warmed on a water bath and, after addition of ammonium oxalate solution, was further warmed until the calcium oxalate was entirely precipitated. The precipitate of calcium oxalate was heated and weighed as calcium oxide.

Magnesium.—The filtrate from the calcium oxalate was concentrated, ammonia added till it reacted strongly alkaline, and sodium citrate and sodium phosphate were added. The precipitate of calcium magnesium phosphate was converted into magnesium pyrophosphate and weighed.

Iron was determined by Neumann's (10) method.

A preliminary qualitative analysis indicated the presence in the soluble ash of amniotic fluid of chlorine, sulfuric acid, phosphoric acid, Na, K, Ca, and Mg, and in the insoluble ash of carbonic acid, sulfuric acid, phosphoric acid, Ca, Mg, and Fe.

The 200 cc. of amniotic fluid examined in Table IV, and those in Tables V and VI were quantitatively analyzed, with results as shown in Table VII.

For a fourth sample, 10, 20, and 170 cc. of amniotic fluid were taken respectively from three women—200 cc. in all—and for a fifth sample, 60,
Table VII.

<table>
<thead>
<tr>
<th>Substance</th>
<th>Soluble ash (1.2063 gm.), gm.</th>
<th>Insoluble ash (0.0322 gm.), gm.</th>
<th>Total ash (1.2385 gm.), gm.</th>
<th>Ash per 100 cc. fluid, gm.</th>
<th>Soluble ash (1.3651 gm.), gm.</th>
<th>Insoluble ash (0.2650 gm.), gm.</th>
<th>Total ash (1.6301 gm.), gm.</th>
<th>Ash per 100 cc. fluid, gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>0.7313</td>
<td>0</td>
<td>0.7313</td>
<td>0.3656</td>
<td>0.7377</td>
<td>0</td>
<td>0.7377</td>
<td>0.3688</td>
</tr>
<tr>
<td>NaCl*</td>
<td>1.2050</td>
<td>0.6025</td>
<td>1.8075</td>
<td>0.9049</td>
<td>1.2154</td>
<td>0.6077</td>
<td>1.8231</td>
<td>0.9134</td>
</tr>
<tr>
<td>SO₃</td>
<td>0.1056</td>
<td>Trace</td>
<td>0.1056</td>
<td>0.0528</td>
<td>0.1490</td>
<td>Trace</td>
<td>0.1490</td>
<td>0.0745</td>
</tr>
<tr>
<td>S</td>
<td>0.0422</td>
<td>&quot;</td>
<td>0.0422</td>
<td>0.0211</td>
<td>0.0592</td>
<td>&quot;</td>
<td>0.0592</td>
<td>0.0289</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.0048</td>
<td>0.0082</td>
<td>0.0130</td>
<td>0.0069</td>
<td>0.0027</td>
<td>0.0094</td>
<td>0.0027</td>
<td>0.0060</td>
</tr>
<tr>
<td>P</td>
<td>0.0056</td>
<td>0.0028</td>
<td>0.0084</td>
<td>0.0041</td>
<td>0.0052</td>
<td>0.0060</td>
<td>0.0052</td>
<td>0.0060</td>
</tr>
<tr>
<td>Na₂O</td>
<td>0.7531</td>
<td>0</td>
<td>0.7531</td>
<td>0.3765</td>
<td>0.7661</td>
<td>0</td>
<td>0.7661</td>
<td>0.3830</td>
</tr>
<tr>
<td>Na</td>
<td>0.5587</td>
<td>0</td>
<td>0.5587</td>
<td>0.2793</td>
<td>0.5685</td>
<td>0</td>
<td>0.5685</td>
<td>0.2842</td>
</tr>
<tr>
<td>K₂O</td>
<td>0.0459</td>
<td>0</td>
<td>0.0459</td>
<td>0.0244</td>
<td>0.0464</td>
<td>0</td>
<td>0.0464</td>
<td>0.0232</td>
</tr>
<tr>
<td>K</td>
<td>0.0408</td>
<td>0</td>
<td>0.0408</td>
<td>0.0204</td>
<td>0.0385</td>
<td>0</td>
<td>0.0385</td>
<td>0.0192</td>
</tr>
<tr>
<td>CaO</td>
<td>0.0030</td>
<td>0.0314</td>
<td>0.0344</td>
<td>0.0172</td>
<td>0.0024</td>
<td>0.0302</td>
<td>0.0024</td>
<td>0.0163</td>
</tr>
<tr>
<td>Ca</td>
<td>0.0021</td>
<td>0.0224</td>
<td>0.0245</td>
<td>0.0122</td>
<td>0.0017</td>
<td>0.0215</td>
<td>0.0017</td>
<td>0.0116</td>
</tr>
<tr>
<td>MgO</td>
<td>0.0008</td>
<td>Trace</td>
<td>0.0008</td>
<td>0.0004</td>
<td>0.0010</td>
<td>0.0002</td>
<td>0.0010</td>
<td>0.0006</td>
</tr>
<tr>
<td>Mg</td>
<td>&quot;</td>
<td>0.0004</td>
<td>0.0004</td>
<td>0.0002</td>
<td>&quot;</td>
<td>0.0006</td>
<td>&quot;</td>
<td>0.0003</td>
</tr>
</tbody>
</table>

* The quantity of NaCl was calculated from the quantity of Cl.

Examine the above figures, we find that the only marked variation is in the amount of sulfuric acid.

To find the relations of the albumins in amniotic fluid to sulfuric acid and other ash, 400 cc. of the fluid obtained from fourteen cases were divided into two equal parts. The one part (A) was dried, ashed, dissolved in water, and diluted up to 200 cc., of which 20 cc. were used for the quantitative determination of sulfuric acid, 50 cc. for phosphoric acid, and 130 cc. for Ca and Mg. The other part (B) was diluted with nine volumes of water, and the albumins were entirely precipitated with common salt and colloidal ferric hydroxide according to Rona and Michaelis (11) and Oppler and Rona. The precipitate was mixed with 500 cc. of water and filtered; this treatment was repeated two more
times. The filtrates were then combined, evaporated, after the addition of a little acetic acid, ashed, and analyzed. In the second part (B) the amount of sulfuric acid evidently was decreased, and the quantity of magnesium also a little diminished. From these facts we may conclude that a part of the sulfuric acid in the ash of amniotic fluid is produced from the sulfur of albumins.

TABLE IX.

<table>
<thead>
<tr>
<th>Amniotic fluid (200 cc.) (A)</th>
<th>Ash per 100 cc. fluid</th>
<th>Amniotic fluid (200 cc.) (B)</th>
<th>Ash per 100 cc. fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
<td>gm.</td>
</tr>
<tr>
<td>SO₃</td>
<td>0.0518</td>
<td>0.0259</td>
<td>0.0227</td>
</tr>
<tr>
<td>S</td>
<td>—</td>
<td>0.0103</td>
<td>—</td>
</tr>
<tr>
<td>P₉O₅</td>
<td>0.0142</td>
<td>0.0071</td>
<td>—</td>
</tr>
<tr>
<td>P</td>
<td>—</td>
<td>0.0030</td>
<td>—</td>
</tr>
<tr>
<td>CaO</td>
<td>0.0252</td>
<td>0.0126</td>
<td>0.0268</td>
</tr>
<tr>
<td>Ca</td>
<td>—</td>
<td>0.0090</td>
<td>—</td>
</tr>
<tr>
<td>MgO</td>
<td>0.0008</td>
<td>0.0004</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mg</td>
<td>—</td>
<td>0.0002</td>
<td>Trace</td>
</tr>
</tbody>
</table>
To discover the relations of the phosphoric acid in the ash of amniotic fluid to albumins and other organic substances in the liquor, samples of the fluid from two women were divided into two parts. One part (A) was used for the quantitative determination of phosphoric acid. The other part (B) was freed from albumins by coagulation; the organic phosphorus substances were removed from the filtrate by extraction with ether, and the phosphoric acid was determined according to Neumann. The two results were nearly identical.

<table>
<thead>
<tr>
<th>TABLE X.</th>
<th>I. Amniotic fluid (80 cc.).</th>
<th>II. Amniotic fluid (100 cc.).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (40 cc.)</td>
<td>B (40 cc.).</td>
</tr>
<tr>
<td>P_2O_5</td>
<td>0.0049</td>
<td>0.0050</td>
</tr>
<tr>
<td>P</td>
<td>0.0021</td>
<td>0.0020</td>
</tr>
</tbody>
</table>

In order to decide whether calcium or magnesium combined with albumins, 100 cc. of amniotic fluid from two cases were divided into two equal parts; one part (A) was used for the quantitative determination of Ca and Mg; the other part (B) was freed from albumins with colloidal ferric hydroxide, and used for the same determination. The second half (B) was but little reduced in the quantity of Mg.

<table>
<thead>
<tr>
<th>TABLE XI.</th>
<th>Amniotic fluid (100 cc.).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A (50 cc.)</td>
</tr>
<tr>
<td></td>
<td>gm.</td>
</tr>
<tr>
<td>CaO</td>
<td>0.0082</td>
</tr>
<tr>
<td>Ca</td>
<td>0.0058</td>
</tr>
<tr>
<td>MgO</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mg</td>
<td>0.0001</td>
</tr>
</tbody>
</table>

To decide whether the amniotic fluid contains ether sulfuric acid, 100 cc. of the fluid were collected from four cases, the albumins were precipitated with colloidal ferric hydroxide, and filtered,
the whole volume being 1,000 cc. From the filtrate, 900 cc. (cor-
responding to 90 cc. of amniotic fluid) were evaporated to 85
cc.; 15 cc. of dilute hydrochloric acid and 15 cc. of 5 per cent
barium chloride solution were added, and barium sulfate was
filtered. The weight of barium sulfate produced here represents
the inorganic sulfuric acid. The filtrate of barium sulfate was
boiled on a water bath until the newly produced barium sulfate was
totally precipitated; then from its weight, the weight of ether
sulfuric acid was calculated, as shown in Table XII.

<table>
<thead>
<tr>
<th>TABLE XII.</th>
<th>Amniotic fluid (100 cc.).</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>gm.</td>
</tr>
<tr>
<td>Inorganic sulfuric acid</td>
<td>SO₃</td>
</tr>
<tr>
<td>Ether &quot; &quot;</td>
<td>SO₃</td>
</tr>
<tr>
<td>Total &quot; &quot;</td>
<td>SO₃</td>
</tr>
</tbody>
</table>

In two experiments 30 cc. of amniotic fluid from two cases each,
were reduced to ash, dissolved in hydrochloric acid, and the quan-
tity of iron was determined by Neumann's method. The results
are given in Table XIII.

<table>
<thead>
<tr>
<th>TABLE XIII.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amniotic fluid (30 cc.). Amniotic fluid (30 cc.).</td>
</tr>
<tr>
<td>gm.</td>
</tr>
<tr>
<td>Fe</td>
</tr>
<tr>
<td>Fe₂O₃</td>
</tr>
</tbody>
</table>

Furthermore, in one case each of the 5th, 6th, 8th, and 9th
months of pregnancy the quantity of sodium chloride was deter-
mained by Volhard’s (12) method (Table XIV).

These values show that there is no marked difference to be
found in the quantity of sodium chloride at different periods of
pregnancy.

Summary.—(1) The amniotic fluid of women at the end of
pregnancy always contains Cl, carbonic acid, sulfuric acid, phos-
phoric acid, Na, K, Ca, Mg, and Fe. (2) The amounts of Cl, phosphoric acid, K, Na, Ca, and Fe are nearly constant. (3) The greatest part of the inorganic salts is sodium chloride, which averages 75.2 per cent of the total ash. (4) A part of the sulfuric acid in the ash comes from the sulfur of albumins and a very small part comes from ether sulfuric acid. Therefore, the quantity of sulfuric acid in the ash depends on the quantity of albumins. (5) The average quantities of the inorganic components are shown in Table XV.

### TABLE XV.

<table>
<thead>
<tr>
<th>Inorganic components</th>
<th>Average gm.</th>
<th>Inorganic components</th>
<th>Average gm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>99.480</td>
<td>P</td>
<td>0.0026</td>
</tr>
<tr>
<td>Solid substances</td>
<td>1.2425</td>
<td>Na₂O</td>
<td>0.3819</td>
</tr>
<tr>
<td>Organic substances</td>
<td>0.4504</td>
<td>Na</td>
<td>0.2534</td>
</tr>
<tr>
<td>Soluble ash</td>
<td>0.7690</td>
<td>K₂O</td>
<td>0.0232</td>
</tr>
<tr>
<td>Insoluble ash</td>
<td>0.0407</td>
<td>K</td>
<td>0.0194</td>
</tr>
<tr>
<td>Total ash</td>
<td>0.8096</td>
<td>CaO</td>
<td>0.0157</td>
</tr>
<tr>
<td>Cl</td>
<td>0.3695</td>
<td>Ca</td>
<td>0.0111</td>
</tr>
<tr>
<td>NaCl</td>
<td>0.6000</td>
<td>MgO</td>
<td>0.0005</td>
</tr>
<tr>
<td>SO₃</td>
<td>0.0432</td>
<td>Mg</td>
<td>0.0003</td>
</tr>
<tr>
<td>S</td>
<td>0.0172</td>
<td>Fe₂O₃</td>
<td>0.0244</td>
</tr>
<tr>
<td>P₂O₅</td>
<td>0.0061</td>
<td>Fe</td>
<td>0.0085</td>
</tr>
</tbody>
</table>
d-Lactic Acid and Sugars in Human Amniotic Fluid.

**Method.**

Amniotic fluid was evaporated on a water bath to one-tenth its volume then poured into ten parts alcohol, and extracted. The alcoholic extract was concentrated, the residue dissolved in a small quantity of water, phosphoric acid added until the solution was strongly acid, and the solution extracted in a Suto extraction apparatus with ether for 24 hours. The brown residue from the ethereal extract was dissolved in water, treated with freshly prepared lead carbonate, heated on a water bath for nearly 30 minutes, and filtered. The filtrate was freed from lead by H₂S, the H₂S removed by vacuum distillation, zinc oxide added to the condensed solution, the mixture boiled on a water bath for 30 minutes, and filtered. The filtrate was decolorized by blood charcoal and the clear solution thus obtained was evaporated until the zinc lactate began to crystallize, when a little alcohol was added. As the white crystals of zinc lactate were not yet quite pure, they were washed several times with absolute alcohol, dissolved in a small quantity of hot water and brought again to crystallization.

It was then assured that the white crystals thus prepared were no other than those of zinc d-lactate by (1) examination of crystal shape, (2) Uffalmann's reaction, (3) Fletcher and Hopkins' reaction, (4) measurement of the specific rotation, (5) quantitative determination of the water of crystallization, (6) elementary analysis.
Experiment 1.—1,000 cc. of clean amniotic fluid collected from fourteen healthy women in labor, were treated as above, giving 0.9 gm. of zinc salt, colorless needles, or rhombic prisms, giving a positive Uffelmann's reaction and Fletcher and Hopkins' reaction.

\[
\left[\alpha\right]_{D}^{0} = \frac{-0.4 \times 100}{2.506 \times 2} = -7.98^\circ.
\]

0.3359 gm. salt lost 0.0433 gm. H₂O on drying at 110° to constant weight. 0.2045 " dry salt gave 0.0679 gm. ZnO.

0.0768 " 0.0834 " CO₂ and 0.027 gm. H₂O.

Experiment 2.—1,000 cc. of yellowish green amniotic fluid collected from twenty cases and mixed with a small quantity of meconium, gave 1 gm. of zinc salt.

\[
\left[\alpha\right]_{D}^{0} = \frac{-0.58 \times 100}{3.646 \times 2} = -7.95^\circ.
\]

0.1567 gm. salt lost 0.0204 gm. H₂O. 0.211 " dry salt gave 0.0707 gm. ZnO.

Experiment 3.—2,000 cc. of amniotic fluid obtained from a case of acute hydramnios at the end of the 8th month of pregnancy, gave 1.917 gm. of zinc salt.

\[
\left[\alpha\right]_{D}^{0} = \frac{-0.66 \times 100}{4.064 \times 2} = -8.08^\circ.
\]

0.5143 gm. salt lost 0.0698 gm. H₂O. 0.112 " dry salt gave 0.0874 gm. ZnO.

0.2054 " " 0.073 gm. H₂O and 0.2187 CO₂.

From the foregoing results it is possible to conclude that d-lactic acid is a constant component of human amniotic fluid.

### TABLE XVI.

<table>
<thead>
<tr>
<th>Zinc salt prepared from human amniotic fluid</th>
<th>Zinc d-lactate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experiment.</td>
<td>1</td>
</tr>
<tr>
<td>Crystals.</td>
<td>Needle-shaped or rhombic prisms.</td>
</tr>
<tr>
<td>Specific rotation ([\alpha]_{D}^{11^0})</td>
<td>-7.98°</td>
</tr>
<tr>
<td>Water of crystallization</td>
<td>per cent</td>
</tr>
<tr>
<td>Zn</td>
<td>12.89</td>
</tr>
<tr>
<td>C</td>
<td>26.65</td>
</tr>
<tr>
<td>H</td>
<td>29.62</td>
</tr>
<tr>
<td></td>
<td>3.86</td>
</tr>
</tbody>
</table>
Quantitative Determination of the d-Lactic Acid in Human Amniotic Fluid.

According to Yoshikawa's (15) method, a known quantity of amniotic fluid, after filtering and centrifuging, was evaporated on a water bath, extracted with about ten parts of alcohol, and filtered. The sediment was washed several times with alcohol, the wash alcohol and the alcoholic extract were combined and concentrated, the residue was dissolved in water made strongly acid by the addition of phosphoric acid, and extracted in a Suto extraction apparatus with ether for 72 hours. The residue of the ethereal extract was dissolved in water, lithium carbonate added, and the mixture heated on a water bath for a half hour and filtered. After decolorizing with blood charcoal, the solution was evaporated and made up to 20 cc. From the observed rotatory angle the amount of lithium d-lactate of d-lactic acid in the amniotic fluid was calculated.

With the same method, d-lactic acid was determined in ten cases which varied from 0.1355 per cent to 0.0336, with an average of 0.0726 per cent.

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>No. of amniotic fluid.</th>
<th>Quantity of amniotic fluid.</th>
<th>Temperature.</th>
<th>Rotary angle (α).</th>
<th>Quantity of lithium lactate.</th>
<th>Quantity of d-lactic acid.</th>
<th>Gm. per 100 cc. fluid</th>
<th>Remarks.</th>
</tr>
</thead>
<tbody>
<tr>
<td>I 63</td>
<td>60</td>
<td>12</td>
<td>−0.03°</td>
<td>0.02098</td>
<td>0.02017</td>
<td>0.0336</td>
<td>End of 10th month, mixed with a little meconium.</td>
<td></td>
</tr>
<tr>
<td>II 92</td>
<td>60</td>
<td>12</td>
<td>−0.050°</td>
<td>0.03506</td>
<td>0.03371</td>
<td>0.0561</td>
<td>10th month.</td>
<td></td>
</tr>
<tr>
<td>III 95</td>
<td>60</td>
<td>12</td>
<td>−0.035°</td>
<td>0.02469</td>
<td>0.02374</td>
<td>0.0395</td>
<td>10th “</td>
<td></td>
</tr>
<tr>
<td>IV 96</td>
<td>120</td>
<td>12</td>
<td>−0.100°</td>
<td>0.07012</td>
<td>0.06742</td>
<td>0.0561</td>
<td>10th “</td>
<td></td>
</tr>
<tr>
<td>V 97</td>
<td>120</td>
<td>12</td>
<td>−0.130°</td>
<td>0.09134</td>
<td>0.08782</td>
<td>0.0761</td>
<td>10th “</td>
<td></td>
</tr>
<tr>
<td>VI 98</td>
<td>60</td>
<td>12</td>
<td>−0.055°</td>
<td>0.03850</td>
<td>0.03700</td>
<td>0.0617</td>
<td>Mixed with meconium, 10th month.</td>
<td></td>
</tr>
<tr>
<td>VII 116</td>
<td>100</td>
<td>25</td>
<td>−0.100°</td>
<td>0.07010</td>
<td>0.06740</td>
<td>0.0674</td>
<td>10th month.</td>
<td></td>
</tr>
<tr>
<td>VIII 118</td>
<td>100</td>
<td>25</td>
<td>−0.190°</td>
<td>0.13407</td>
<td>0.12890</td>
<td>0.1289</td>
<td>35th week of pregnancy.</td>
<td></td>
</tr>
<tr>
<td>IX 122</td>
<td>100</td>
<td>25</td>
<td>−0.110°</td>
<td>0.07720</td>
<td>0.07430</td>
<td>0.0743</td>
<td>10th month.</td>
<td></td>
</tr>
<tr>
<td>X 128</td>
<td>100</td>
<td>25</td>
<td>−0.200°</td>
<td>0.14090</td>
<td>0.13550</td>
<td>0.1355</td>
<td>10th “</td>
<td></td>
</tr>
<tr>
<td>Average</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.0726</td>
</tr>
</tbody>
</table>
Amniotic Fluid

Sugars.

Gürber and Grünbaum (16) found some fruit sugar in the amniotic fluid of cows, sheep, and swine, but could not find even a trace of sugars in the fluid of ten women examined in the same way. Grünbaum (17) states that the amniotic fluid of cats and dogs is free from fruit sugar. However, Ludwig (18) found 0.3 gm. of sugar in 4,000 cc. of amniotic fluid obtained by artificial puncture of fetal membranes of a diabetes patient, whose urine contained about 3.8 per cent sugar.

In order to decide whether human amniotic fluid is surely free from sugars, an aqueous solution, after being freed from lactic acid, was neutralized with calcium carbonate, and filtered. The voluminous precipitate of calcium phosphate was then mixed with water in a porcelain mortar and filtered again by suction. The filtrates were combined and concentrated to 100 cc. and used for the following examinations: Trommer's and Nylander's reactions were negative; the phenylhydrazine reaction was negative; and finally, the solution was optically inactive. The results, therefore, of these experiments confirm the statements of Gürber and Grünbaum that sugars are not a constant component of human amniotic fluid.

Summary.—(1) The d-lactic acid is a constant component of human amniotic fluid and amounts to 0.0726 per cent on an average. (2) Sugars are not to be found in human amniotic fluid.

Allantoin and Organic Bases in Human Amniotic Fluid.

According to Gusserow and Hermann, the urine of healthy bodies always contains a small quantity of allantoin, while the urine of pregnant women contains more. Wiechowski (19), however, states that he could find no allantoin in human urine, in 200 cc. of urine of a 1½ months old suckling baby, nor in the urine of a new-born child at its first day; furthermore, allantoin was absent in the amniotic fluid of two pregnant women. It is still an open question whether allantoin is a component of human amniotic fluid; the amino-acids or organic bases of human amniotic fluid have not been investigated.

In the present investigation, 3,064 cc. of amniotic fluid were precipitated with basic lead acetate and filtered. The filtrate was freed from lead, concentrated in vacuo at 50° to about 150 cc., and filtered. The filtrate was diluted with water to 200 cc., concentrated sulfuric acid added until the latter amounted to 5 per cent in volume, and the solution filtered. The filtrate was treated with 20 per cent phosphotungstic acid solution and, after standing for a night, filtered, giving a reddish white precipitate (A),
Organic Bases.—The precipitate (A) was washed several times with 5 per cent sulfuric acid, then mixed with barium hydroxide in a porcelain mortar, and diluted with water. Carbon dioxide was passed into this mixture, the barium carbonate filtered, and the filtrate distilled in vacuo to 50 cc.; addition of dilute nitric acid and silver nitrate gave a small quantity of brown precipitate (I), purine bases, and a filtrate (II).

Purine Bases.—Precipitate I gave a negative test for purine bases.

Hexone Bases and Choline.—Filtrate II was divided into two portions with silver nitrate and barium hydroxide; the resulting precipitate (III) was washed several times with dilute barium hydroxide and used for the preparation of histidine and arginine, while the filtrate (IV) was used to test for choline and lysine. Using the ordinary tests and methods of separation, histidine was demonstrated by a positive Pauly diazo reaction; a picrate was obtained as fine yellow, silken needles, where one would expect to find arginine, but its melting point was 182°. Lysine was isolated as the picrate, orange-yellow needles, melting at 252°. Choline was absent.

Allantoin.—Allantoin was isolated from filtrate B according to Wielchowski (20). (The filtrate from the mercury acetate was used to prove the presence of amino-acids.) The material obtained from the mercury acetate gave a positive Eppinger (21) glyoxylic acid reaction, and gave oxalic acid when heated with 15 per cent sodium hydroxide (Salkowski, 22). Thus, while pure crystals of allantoin could not be isolated, a very small quantity of allantoin probably is to be found in human amniotic fluid.

Amino-Acids.—The filtrate from the mercury acetate-allantoin precipitate was freed of Hg and found to contain 0.3 gm. N. The presence of amino-acids was tested for according to Ignatowski (23), but with negative results.

Summary.—The results of the experiment, which was performed with 3,000 cc. of amniotic fluid are as follows: (1) Neither purine bases, choline, nor monoamino-acids are to be found in amniotic fluid. (2) Histidine, lysine, and allantoin are all present in very small quantity. (3) The existence of arginine is still undecided.

Albumins, Urea, Uric Acid, Creatine, and Cholesterol in Human Amniotic Fluid.

Albumins.

Koettnitz (24) found propeptone in the amniotic fluid in two cases in pregnancy of 2½ and 2½ months, respectively; peptone and propeptone in four cases in the 10th month, and peptone
Amniotic Fluid

alone in one case in the 10th month; from these facts he concluded that the amniotic fluid is used as nourishment for the fetus at the beginning of pregnancy, and has the same use, though in much less degree, at the end of pregnancy. Weyl (25) confirmed the presence of mucin in the amniotic fluid of two women in hydramnios and determined the quantities as 0.1 and 0.2 per cent.

Experiment 1.—37 cc. of amniotic fluid were centrifuged, filtered by suction, treated with an equal volume of a saturated solution of ammonium sulfate, and filtered after standing over night. The filtrate heated on a water bath, gave a heavy precipitate; the filtrate of this precipitate gave no biuret reaction. The precipitate, obtained by the addition of ammonium sulfate solution, was washed several times with a half saturated solution of ammonium sulfate, until its filtrate gave no more albumin reactions; when dissolved in a small quantity of water and heated on a water bath at more than 70°, the solution became white and cloudy; when boiled and filtered, the transparent filtrate showed a very weak biuret reaction.

Experiment 2.—60 cc. of nearly clear and yellowish amniotic fluid were diluted with an equal volume of water and filtered by suction. When mixed with a few drops of acetic acid, the filtrate showed a white cloudiness, which could not be redissolved by the addition of excess acetic acid. After standing over night, the slight precipitate was filtered and washed with very dilute acetic acid. The precipitate, boiled with dilute sulfuric acid, gave a solution with positive Trommer’s reaction. The filtrate of the precipitate produced by the addition of acetic acid was neutralized with sodium carbonate, and upon adding an equal volume of a saturated solution of ammonium sulfate, gave a cloudiness. The filtrate was heated on a water bath and filtered from the coagulated albumins, but this filtrate gave no sign of biuret reaction.

The precipitate produced by the addition of ammonium sulfate was washed with a half saturated solution of ammonium sulfate, dissolved in a small quantity of water and heated on a water bath at more than 70° when the solution became white and cloudy. When boiled and filtered, the transparent filtrate still showed a very weak biuret reaction.

Experiment 3.—According to Hohlweg and Meyer (26), a mixture of a volume of 1 per cent acetic acid with an equal volume of 5 per cent monopotassium phosphate solution was added to 80 cc. of amniotic fluid until it reacted acid against Congo red paper, but neutral against litmus paper; then an equal volume of a saturated sodium chloride solution was added; this mixture was boiled on a water bath to coagulate the albumins, and filtered. The filtrate obtained thus gave no biuret reaction.

In Experiment 2, the appearance of the glucoprotein, which was produced by the addition of acetic acid and was not soluble
in excess acetic acid, confirmed the existence of mucin in amniotic fluid; Experiment 1 demonstrated that albumin and a little globulin were present in amniotic fluid, but that no peptones were present. Experiment 3 also demonstrated that peptones were absent. But whether the very weak biuret reaction in the filtrate of the coagulable albumins in Experiments 1 to 3 was due to the presence of albumoses could not be determined.

Quantitative Determination of Coagulable Albumins.

Prochownick (1) found 0.71 to 0.06 per cent coagulable albumins in the amniotic fluid of eleven women; Ahlfeld (27) states that the amniotic fluid of seven out of twenty women contained 25 to 50 volume per cent albumins, and from this fact he argued that amniotic fluid must be a nourishment for the fetus; however, his argument is not adequate as the following results of other authors indicate. According to Schroeder (2), the amount of albumins in the amniotic fluid stands in a close relation to the quantity of hemoglobin in the maternal blood; in 50 cases it amounted to 0.03 to 0.308 per cent. In the mature fetus, it averaged 0.1081 per cent, and 0.1578 per cent in the immature fetus. In Döderlein’s (6) experiments with fifteen cows, the quantity of albumins in the amniotic fluid amounted to 0.042 to 0.455 per cent; it increased gradually as the pregnancy proceeded. Jacqué found 0.023 to 0.058 per cent albumins in the amniotic fluid of the sheep fetus, when less than 14 cm. in length, and 0.1 per cent albumins when over 30 cm. in length.

In the present experiments, human amniotic fluid was first filtered through fourfold gauze, then centrifuged, and filtered by suction. Thus prepared, the fluid was diluted with three parts of water, sodium chloride added to make 1 per cent, the solution made weakly acid with 1 per cent acetic acid, and boiled on a water bath. This completely coagulated the albumins which were filtered through a tared ashless filter, washed with warm water, then with alcohol, and finally with ether. Thus treated, the albumins were dried on the filter at 110° and weighed. Since the coagulated albumins thus obtained usually contained more or less ash, this ash was determined, and from it the weight of pure coagulable albumins calculated. The value obtained by the above method is the sum of the albumin, globulin, and mucin, but this may be considered coagulable albumins in a narrow sense, since the quantity of mucin in the amniotic fluid is very small.

The quantity of the coagulable albumins in the amniotic fluid of fifteen women at the end of pregnancy varied from 0.092 to
0.421 per cent, with an average of 0.226 per cent. In two cases of the 5th and 6th months of pregnancy, respectively, the amount of coagulable albumins is not markedly different from the amount at the end of pregnancy. The results of these experiments are shown in Table XVIII.

### Table XVIII

<table>
<thead>
<tr>
<th>No. of experiment</th>
<th>No. of amniotic fluid</th>
<th>Quantity of amniotic fluid cc.</th>
<th>Coagulable albumin gm. per 100 cc. fluid</th>
<th>Remarks</th>
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<td>0.0421</td>
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</tr>
<tr>
<td>2</td>
<td>85</td>
<td>5</td>
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<td></td>
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<td>3</td>
<td>86</td>
<td>10</td>
<td>0.0160</td>
<td></td>
</tr>
<tr>
<td>4</td>
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</tr>
<tr>
<td>5</td>
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<td>15</td>
<td>115</td>
<td>10</td>
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<td>Average</td>
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<td></td>
<td>0.0384</td>
<td>0.384</td>
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<tr>
<td>16</td>
<td>191</td>
<td>10</td>
<td>0.0384</td>
<td>5th month, artificial abortion for consumption.</td>
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<tr>
<td>17</td>
<td>227</td>
<td>10</td>
<td>0.0416</td>
<td></td>
</tr>
</tbody>
</table>

Quantitative Determination of Urea and Distribution of Nitrogen.

Although Scherer, Mack, and Calberg denied the existence of urea in the amniotic fluid, Woeler discovered it by exact investigation. Later, other investigators have given the following values: Funke, 0.38 per cent; Letzmann, 0.05 per cent; Majewski, 0.34 to 0.42 per cent; Beale (28), 0.35 per cent; Siewert (29), 0.0352 per cent; Winckel (30), 0.086 to 0.42 per cent; Gusserow (31) 0.14 to 0.35 per cent. Prochownik (7) determined the quantity of urea as nitric urea in two cases in the first half of pregnancy and also in eleven cases at the end of pregnancy, and found 0.0155 to 0.034 per cent; since the amount of urea was small at the beginning and greater at the end
of pregnancy, he concluded that urea in the amniotic fluid is secreted from
the skin and the kidneys of the fetus, and that the fluid itself is a product
of the fetus. Schöndorff (32) stated that the amount of urea in the amni-
otic fluid (0.0611 per cent) is about the same as that in the blood and the
milk.

In the present investigations, the ammonia was determined by
the method of Brugsch and Schittenhelm (33); Marshall's
(34) method was used for the determination of urea, and Kjel-
dahl's for the total nitrogen. The albumin N was determined in
the precipitate obtained with colloidal ferric hydroxide. The
amount of ammonia varied from 0.0051 to 0.0013 per cent, with

\[
\begin{array}{cccccccc}
\text{No. of} & \text{No. of} & \text{Ammonia} & \text{Urea} & \text{Total N.} & \text{Albumin N.} & \text{Rest N.} & \text{Ammonia N.}^* & \text{Urea N.} & \text{Remaining N.} \\
\text{experiment} & \text{Amniotic Fluid} & \text{per cent} & \text{per cent} & \text{per cent} & \text{per cent} & \text{per cent} & \text{per cent} & \text{per cent}} \text{per cent} \\
1 & 112 & 0.0040 & 0.0276 & \\
2 & 113 & 0.0023 & 0.0570 & \\
3 & 115 & 0.0015 & 0.0519 & \\
4 & 117 & 0.0023 & 0.0666 & \\
5 & 122 & 0.0017 & 0.0504 & \\
6 & 132 & 0.0013 & 0.0297 & \\
7 & 143 & 0.0044 & 0.0204 & \\
8 & 211 & 0.0028 & \\
9 & 219 & 0.0051 & \\
10 & 227 & 0.0017 & 0.1950 & 0.1036 & 0.0866 & 0.0168 & 0.0014 & 0.0091 & 0.0063 \\
11 & 232 & 0.0034 & 0.0300 & \\
12 & 237 & 0.0051 & 0.1800 & 0.0588 & 0.0441 & 0.0147 & 0.0042 & 0.0084 & 0.0021 \\
13 & 243 & 0.0025 & 0.1950 & 0.0518 & 0.0338 & 0.0182 & 0.0021 & 0.0091 & 0.0070 \\
14 & 247 & 0.0034 & 0.0255 & \\
15 & 248 & 0.0029 & 0.1870 & 0.0770 & 0.0635 & 0.0135 & 0.0024 & 0.0087 & 0.0024 \\
16 & 250 & 0.0029 & 0.0174 & 0.0840 & 0.0672 & 0.0105 & 0.0024 & 0.0001 & 0.0083 \\
\text{Average} & & 0.0029 & 0.0323 & 0.0750 & 0.0590 & 0.0159 & 0.0025 & 0.0083 & 0.0052 \\
\end{array}
\]

* The ammonia N corresponds to 15.7 per cent of the so called rest N, and
the urea N corresponds to 52.2 per cent of the rest N.
an average of 0.0029 per cent; the urea varied from 0.0066 to 0.0174 per cent, with an average of 0.0323 per cent. Five experiments on the distribution of N gave average values of: albumin N, 0.059 per cent; rest N, 0.0159 per cent; ammonia N, 0.0025 per cent; urea N, 0.0083 per cent. These results are seen in Table XIX.

**Uric Acid.**

653 cc. of amniotic fluid were freed from coagulable albumins by boiling with dilute acetic acid, and then by adding 5 per cent monopotassium phosphate solution and boiling. The filtrate was then tested for uric acid by Schroeder's (35) method. The resulting silver compound was freed from silver, the residue extracted with a dilute sodium carbonate solution, the solution treated with acetic acid, and evaporated. After a day, some amorphous reddish sediment separated; this was insoluble in cold water, but easily dissolved upon warming and separated again upon cooling. A part of this sediment gave a marked murexide reaction, but there was not enough for an elementary analysis. A second experiment gave negative results. Uric acid in small quantity is contained in the amniotic fluid during confinement.

**Creatine and Creatinine.**

Gönner (36) states that creatine and creatinine should be contained in the amniotic fluid, but that they have not been secured experimentally.

In one experiment, the filtrate from the uric acid precipitate (silver and magnesia) was freed from silver by hydrochloric and nitric acids and used for the preparation of creatine according to Neubauer and Salkowski's (37) method, with negative results, neither creatine nor creatinine being found. In another experiment, 420 cc. of pure amniotic fluid, treated in the same way, showed the presence of creatine but creatinine was absent. Amniotic fluid during confinement contains creatine in very small quantity, but no creatinine.

**Hippuric Acid.**

Until the present experiments, no investigation has been made for hippuric acid as a component of the amniotic fluid.
526 cc. of amniotic fluid were precipitated with lead acetate, the lead in the filtrate was removed with H₂S, the filtrate concentrated in vacuo to a syrup, and extracted with alcohol; the residue was dissolved in a small quantity of hot water, acidified with dilute hydrochloric acid and extracted with acetic ether. This extract was washed with water, and, upon evaporation, gave some brown residue which was extracted with petroleum ether. The insoluble part contained no hippuric acid; two other similar experiments were likewise negative. Hippuric acid is not a component of human amniotic fluid.

Cholesterol.

Göunner (36) states that the amniotic fluid contains cholesterol, but no experimental investigation of it could be found in the literature.

For these experiments, 905 cc. of amniotic fluid were extracted with ether in a Suto extraction apparatus for 48 hours, the extract was saponified with dilute alcoholic potassium hydroxide, the residue treated with 20 cc. of hot water, and after cooling, extracted with ether three times. The residue was extracted with petroleum ether, and the soluble portion dissolved in a mixture of ether and alcohol, and the solution allowed to evaporate spontaneously. White, mica-like, glossy, squamiform crystals were obtained; examined under a microscope, they were colorless, very thin, rhombic or irregularly square tablets, sometimes broken at a corner, and piled closely one on top of another. They melted at 146°, were optically active, and gave positive Salkowski’s, Liebermann and Burchard’s, and Schiff’s reactions. From this, it is evident that the substance obtained by extraction with ether from human amniotic fluid is cholesterol, and the cholesterol is beyond a doubt a component of human amniotic fluid.

Summary.—(1) The human amniotic fluid at the end of pregnancy always contains coagulable albumins (an average of 0.226 per cent, inclusive of mucin); albumin is its greatest constituent, but globulin is present in traces; the amount of albumins in the amniotic fluid in the first half of pregnancy is not remarkably different from that at the end. (2) Mucin is also contained, but in quantity too small to determine; peptone and albumoses are
not found. (3) Ammonia and urea are constant components; the amount of ammonia is, on an average, 0.0029 per cent; the amount of urea, on an average, is 0.0323 per cent, and both together furnish about 70 per cent of the so called rest N. (4) Uric acid and creatine, in very small amounts, are present, but neither creatinine nor hippuric acid is found. (5) Cholesterol is a component of human amniotic fluid.

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Doko Uyeno

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