Interest in the amino-acids of the placenta has been stimulated by recent agitation concerning the manner in which the organ performs its physiological function, as well as by the part attributed to it, by some investigators, in the production of certain pathological conditions, more particularly in eclampsia. We have not hoped to solve either of these mooted questions, but we have felt that analysis of the organ by the newer methods of protein hydrolysis would be valuable in contributing to our knowledge of its fundamental composition, and perhaps in affording a logical basis for subsequent study of its physiology and pathology.

The placenta possesses an unique function and unusual importance; upon it depends the successful issue of every human pregnancy. Interpolated between the mother and the foetus, it is so constructed as to permit free passage of the blood of each without any possibility of the two commingling. Such an arrangement is not needed in species in which the egg, supplied with ample material for nourishment, leaves the mother before embryonic development begins, and only becomes necessary when the growth of the offspring occurs within the mother. Through the mediation of this organ, food reaches the foetus, and the waste products of its metabolism are returned to the mother.

A review of the architecture of the placenta will make this mechanism clearer. The placenta is a flat, more or less round structure, some 20 cm. in diameter and 2 to 3 cm. thick. Its weight
Amino-Acids in the Placenta

averages approximately 500 grams, but varies with the size of the child, to which under normal conditions it bears a ratio of 1:6. Its two surfaces are designated as maternal and foetal, and are easily distinguishable, in that the umbilical cord is attached to the latter. Two greyish white, closely adherent membranes extend from the periphery of the placenta, and, unless artificially separated, give the impression of a single structure. The inner one, the amnion, encloses the amniotic fluid which surrounds the foetus during pregnancy; while the outer one, the chorion, is applied to the wall of the uterus. However, except for that portion of the latter which is specialized to form the placenta, these membranes have no interest in the present connection.

Microscopic study has demonstrated that very little maternal tissue is cast off with the placenta, namely a layer 1 to 2 mm. in thickness covering the maternal surface. The bulk of the organ is composed of delicate finger-like processes, the chorionic villi, which dip down into lakes of the mother's blood. The villi themselves are derived from foetal tissue, and enclose small blood vessels which only communicate with the large arteries and vein of the umbilical cord, and therefore, contain foetal blood. Between the two circulations, maternal and foetal, there is always interposed the solid wall of the villus, consisting of two strata, one epithelium, the other connective tissue. The outer of these, the epithelial, is continually bathed in the mother's blood. The connective tissue layer lies beneath the epithelial, which it supports, and in addition supplies a scaffolding that preserves the proper relations of the blood vessels, and maintains the form of the villus.

No channels run through the wall of the villus which might permit a direct means of communication between the blood of the mother and that of the foetus. All substances in transit from one circulation to the other must pass through both the epithelium and the connective tissue which compose the walls of the villi. Consequently, the part the villi play in the process has long been a prominent question, and one not yet answered satisfactorily. Two possibilities have been suggested; one that the wall is inactive, allowing passage in accord with the principles of osmosis and diffusion, the other that it is active, causing chemical changes in at least some of the material which passes through it. Definite
proof has been adduced by Zuntz and his pupils that gaseous bodies and an aqueous solution of sodium chloride pass from one circulation to the other in accord with the laws of physical chemistry. That a different process is concerned in the transmission of other substances would seem likely; and accordingly a number of investigators have brought forward experimental data which they believe indicate a digestive function in the placenta. A similar conclusion was reached by William Harvey in 1651 upon purely theoretical ground.

*Previous Investigations.* The simplest analyses of the placenta have been concerned with the relative amount of water and of dried substance entering into its composition. The technique of these determinations has varied since some of the investigators have included the foetal blood in their material, and others have not. In either event, preparation of the material began with stripping off the umbilical cord, the amnion, and the membranous portion of the chorion as far as the periphery of the placenta. Following this, where it was not desired to remove the foetal blood, as in the analyses of Gaube and others, the organ was cut in pieces and dried to a constant weight. Sfameni and Grandis expressed as much blood as possible from the organ by squeezing it between the hands. Higuchi, very recently published two series of analyses, in one of which the material was unwashed, while in the other the foetal blood was removed by the perfusion of normal salt solution through the blood vessels. The fluid was injected by way of the umbilical vein, and escaped from the two umbilical arteries; about 12 liters of the solution were used for each placenta, but even after this, the washings were not perfectly clear.

The results, tabulated below, indicate that approximately 83 to 85 per cent of the unwashed placenta consists of water. An unusually large deposition of calcium salts increases the relative

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2 Harvey: *Exercitationes de generatione animalium*, London, 1651.
4 Sfameni: *Arch. ital. de biol.*, xxxiv, p. 216, 1900.
amount of solids. Thus, in calcareous placentae, Taltavall and Gies found 80.25 per cent of water and 19.75 per cent of solids which were distributed as follows: 18.09 per cent organic and 1.66 per cent inorganic matter.

**Water, Solids, and Ash in the Human Placenta.**

<table>
<thead>
<tr>
<th>AUTHOR</th>
<th>WATER per cent</th>
<th>SOLIDS per cent</th>
<th>ASH per cent</th>
<th>REMARKS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gaube...........</td>
<td>85.33</td>
<td>14.16</td>
<td></td>
<td>Unwashed placenta: Male children</td>
</tr>
<tr>
<td>Gaube...........</td>
<td>85.50</td>
<td>13.88</td>
<td></td>
<td>Unwashed placenta: Female children</td>
</tr>
<tr>
<td>Taltavall and</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gies.............</td>
<td>82.84</td>
<td>17.16</td>
<td>1.15</td>
<td>Unwashed placenta</td>
</tr>
<tr>
<td>Higuchi..........</td>
<td>84.90</td>
<td>15.09</td>
<td>0.88</td>
<td>Unwashed placenta</td>
</tr>
<tr>
<td>Sfameni..........</td>
<td>83.69</td>
<td>16.32</td>
<td>0.87</td>
<td>Blood squeezed out</td>
</tr>
<tr>
<td>Grandis..........</td>
<td>83.89</td>
<td>16.09</td>
<td>1.07</td>
<td>Blood squeezed out</td>
</tr>
<tr>
<td>Higuchi..........</td>
<td>88.65</td>
<td>11.35</td>
<td>0.71</td>
<td>Placenta perfused with salt solution</td>
</tr>
<tr>
<td>Grandis..........</td>
<td>88.80</td>
<td>11.20</td>
<td>0.795</td>
<td>Placenta perfused with salt solution</td>
</tr>
</tbody>
</table>

It is evident that chemical knowledge of the placental tissue itself cannot be secured unless the blood is removed and, on this account, the results obtained by Higuchi and by Grandis where the organ had been perfused with salt solution, offer the best idea of its composition. When the material for analysis has been so prepared, the relative amount of solids is perceptibly decreased and indicates that almost nine-tenths of the organ is water. Higuchi considers that his technique accounts in some measure for the unusually high water content, since some soluble material was carried out in the stream of salt solution passed through the organ about thirty times. On the other hand, it is possible that the tissue absorbed water. Although these conditions may have had some influence, it can be shown they are not entirely responsible for the high water-content of the organ.

Sfameni found that a given weight of foetal blood contains more solids than an equal weight of placenta. From this it follows that the retention of blood in the organ would increase the amount

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of solids there, and diminish relatively the amount of water. By way of contrast, Sfameni states that the percentage of water in the placenta, foetal blood and adult blood is respectively 83.68, 78.5, and 77.3 per cent.

Ash. Several attempts have been made to find a characteristic difference in the composition of the placenta according as the child was a male or female. The results are unconvincing. Gaube¹ states that whereas the minerals in the bodies of boys are in excess in those of girls, the opposite condition prevails in the placentae. In his analyses the placentae of males yielded an average of 2.164 grams, and those of females 2.255 grams of inorganic matter. Such figures are not sufficiently different to warrant the conclusion Gaube drew; moreover, from his own tables it appears there are many exceptions to the rule. The quantity of inorganic material which Gaube found in the placenta is about one-half that recorded by other investigators.

The results given in the foregoing table indicate that the ash is approximately one per cent of the total weight of the fresh placentae. The greater portion of this is soluble in water, according to Sfameni, who found the relation of the soluble to the insoluble ash as 6 to 1. Higuchi recently finds this relation, 4 to 3.

Obviously, the dried material obtained by driving off all the water would be relatively richer in minerals than the fresh placenta. Taltavall and Gies obtained 6.89 per cent of inorganic matter in dried normal placentae and 8.46 per cent in dried calcareous placentae.

The qualitative study of the placental ash has not been undertaken with sufficient thoroughness to yield reliable data. Gaube's analyses, which were the first reported, aimed at greater completeness than has since been attempted, but his methods were not refined, and his conclusions have never been corroborated.

Grandis² emphasizes the large amount of phosphates in the placenta, which he estimates as 33.46 per cent of the inorganic matter present; the phosphoric acid is in combination with calcium, magnesium and iron. His analyses of the ash also yield 11.4 per cent of chlorine; 24.9 per cent of sodium; and 6.57 per cent of

¹ Gaube: loc. cit.
² Grandis: loc. cit.
potassium. An excess of sodium over potassium is unusual in human tissue; the only analogy, according to Grandis, occurs in the blood serum.

Very recently, Higuchi has estimated the total phosphorous and total sulphur in the placenta. These analyses indicate that the fresh, unwashed placenta contains 0.14 per cent of phosphorus and 0.12 per cent of sulphur; whereas, the corresponding figures with the foetal blood removed are 0.08 per cent and 0.06 per cent.

The Organic Constituents of the placenta have received more attention than its mineral matter but, notwithstanding, we have not, as yet, a clear idea of its organic composition. Work in this direction has been stimulated by interest in the toxaemias of pregnancy, for which a placental origin has been suggested. Those who favor this hypothesis believe that the toxins are of an organic nature.

Carbohydrates. The first organic compound to be demonstrated in the placenta was glycogen; Claude Bernard identified it there in 1859, and subsequent investigators have confirmed his observation. With appropriate technique, it is now possible to localize the deposits of glycogen. Thus, applying microchemical methods, Driessen has shown that in the mature human placenta glycogen is practically confined to the decidua. It is stored most abundantly in the neighborhood of the maternal blood vessels, and in the zone where the foetal and maternal elements commingle, that is, toward the surface of the decidua: very small amounts may be demonstrated at the periphery of the villi. Driessen has also noted that the amount of glycogen in the human placenta decreases as pregnancy advances. Analogous changes have been observed in the rabbit placenta by Lockhead and Cramer.

Accurate, quantitative estimations of the glycogen cannot be secured by microchemical methods; to determine this, the glycogen must be extracted. That procedure was undertaken by Moscati, who found that the placenta at full term contains from 2.5 to 3 grams of glycogen or from 0.49 to 0.58 per cent of its gross

1 Higuchi: loc. cit.
weight. Correct values are only obtained when the placenta is subjected to analysis immediately after its expulsion from the uterus since the glycogen-content rapidly diminishes from this moment. About one-half disappears within twenty minutes, and at the end of twenty-four hours no trace of it remains. Possibly, that explains the low figure obtained by Higuchi who found the glycogen in the placenta equivalent to 0.032 per cent of the weight of the organ.

The disappearance of glycogen from the placenta may be due to one of two causes, namely contamination of the organ with bacteria; or the presence of an enzyme normally located there. The latter view is favored by Moscati, Opocher¹ and by Bergell and Liepman.² Moscati has found a ferment in the placenta which hydrolyzes glycogen and which bears a stronger resemblance to the enzyme found in muscle than to that in the liver. Opocher believes that the action of the ferment is reversible since he observed an increase in the amount of glycogen when the placenta was allowed to soak in a solution of grape sugar. On the other hand, Merletti³ has passed a solution of glucose through the vessels of the placenta and noted that the returning fluid was poorer in sugar than the original solution. He regards a simple diffusion as impossible here since sodium phosphate perfused under similar conditions is returned quantitatively. Santi and Acconci⁴ repeated the experiments of Merletti but were unable to verify his results.

Fats. Upon histological evidence the presence of fat in the placenta has long been admitted and Hofbauer⁵ has recently studied its distribution very carefully by means of alcanna, osmic acid and soudan III. Hofbauer states that he was able to follow the stained globules passing through the walls of the villi toward the foetal circulation. The quantitative analyses of Higuchi, the only ones thus far published, indicate 0.846 per cent of fat in the unwashed and 0.535 per cent of fat in the washed placenta.

¹ Opocher: Annali di ostetr. e ginec., ii, p. 737.
⁴ Santi and Acconci: Ginecologia, p. 311, 1904.
Higuchi also found 0.899 and 0.504 per cent of lecithin in the unwashed and washed tissue respectively.

**Nitrogenous Compounds.** Higuchi determined the total nitrogen (Kjeldahl) in the placenta as 2.226 per cent of the gross weight of the organ with foetal blood included. He found 1.331 per cent of nitrogen when the blood had been washed out. He tacitly assumes that all the nitrogen is present in the form of protein, for he multiplies the nitrogen content by 6.25 and states that the albumen of the unwashed and the washed placenta is 14.16 per cent and 8.32 per cent, respectively. Clearly, the calculation is inexact since a number of other nitrogenous bodies are present.

Nucleoprotein is the only albuminous body the isolation of which has been attempted from the placenta. Cocchi assumed that the lesions in eclampsia are associated with coagulation of the mother's blood, and that this was due to the entrance of placental nucleoprotein into her circulation. Accordingly, in 1901, he sought to isolate this compound from eclamptic placentae, employing both the method of Wooldridge and that of Halliburton for this purpose. He considered that the substances obtained by these methods were identical; though the first gave a larger yield and was, therefore, permanently adopted. A small dose of the material thus obtained was fatal to rabbits; whereas, normal placentae treated in the same fashion furnished a material of very uncertain action and proved harmless in three of four experiments. Although the eclamptic material gave some of the color reactions for protein, the evidence adduced is insufficient to establish its identity. Thus, its composition was studied only with regard to phosphorus; two analyses yielded 1.839 per cent and 0.734 per cent respectively, facts which speak against the compound being a homogeneous one. Similarly, Bottazzi sought a nucleoprotein in the placenta, but confined his study to the normal organ. Here again it is uncertain whether the investigator was dealing with a compound or a mixture.

The most recent attempt to isolate nucleoprotein from the placenta was made by Kikoji, who employed the method of Neuman.

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1 Cocchi: *Lo sperimentale, Arch. d. biol. normale e patologica*, Firenze lv, p. 503, 1901.
Kikoji obtained a substance which corresponded in its properties with the nucleoprotein of the thymus gland, and on decomposition yielded humin substances, levulinic acid, cytosin, thymin, guanin, xanthin, and hypoxanthin. Its elementary composition, however, was not constant.

Kikoji\(^1\) in collaboration with Higuchi first isolated from the placenta the same purin bases that he later found in the nucleoprotein. Rielander\(^2\) has also isolated uracil from the placenta and considers it a decomposition product of the nucleoprotein.

In 1901, Mathes\(^3\) demonstrated albumoses in nine out of ten normal placentae by means of Devoto's method. His finding has been confirmed by Hofbauer\(^4\) and by Basso.\(^5\) Hofbauer believes that the albumoses result from the action of a proteolytic ferment which functions during life and is concerned with the passage of protein from mother to child. Having failed to find amino-acids in the fresh placenta, he believes digestion goes no further than the albumose stage.

The view that the splitting of protein is a normal function of the placenta has been endorsed by Ascoli\(^6\) on very different grounds. This investigator employed the precipitin reaction, and found, after injecting egg albumen into a pregnant rabbit, that the activity of the maternal and the foetal blood in causing precipitation of a specific serum is not identical. He concluded, therefore, that the albumen injected into the mother was modified as it passed to the child. Savaré\(^7\) agrees that a proteolytic ferment exists in the placenta, though its presence does not prove that the organ is other than a permeable membrane, for the enzyme may only have to do with the life processes of the placental cells.

Following Salkowski's\(^8\) demonstration that various organs of the body will digest themselves when kept at 40° C. in sterile

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\(^1\) Kikoji and Higuchi: *Zeitschr. f. physiol. Chem.*, lii, p. 401, 1907.
\(^4\) Loc cit.
\(^7\) Savaré: *Hofmeister's Beiträge*, ix, p. 141, 1907; *Ginecologia, Firenze*, iv, p. 34, 1907.
vessels, it was shown by Mathes that the placenta contains an autolytic enzyme. Subsequently a number of investigations have been made to determine the nature of the enzyme as well as of the end products resulting from its action. Thus, Ascoli, Bergell in collaboration with Liepman and Falk have noted that the enzyme is “heterolytic” as well as “autolytic.” It will split protein and peptones, though its action on gelatin and fibrin is very weak. Its analogy to trypsin is strong according to some investigators; while others consider that the enzyme is erepsin.

Dreyfus has compared the “nitrogen distribution” in the fresh placenta and in the placenta after a week of autolysis. In the filtrate from the fresh material he finds that 4.8 per cent of total nitrogen is present as ammonia, 68.2 per cent as diamino compounds, and 46.2 per cent as monamino compounds; whereas, in the autolysed organ a corresponding analysis yields 9.2 per cent ammonia, 40 per cent diamino compounds and 61.3 per cent monamino compounds. Dreyfus infers there is a “desamidating ferment” in the placenta.

Leucine and tyrosine have been recognized as end products of placental autolysis. Mathes discovered them in a placenta allowed to undergo autodigestion for a period of five and a half months. In repeating this experiment, Basso recognized these monoamino acids through the appearance of the crystals, and substantiated the presence of tyrosine by obtaining a positive Millon reaction. This investigator noted more leucine than tyrosine, though he does not mention having separated the two substances and gives no analyses to establish their identity.

Thus far, no amino-acid has been isolated from the placenta. Rielander sought two of the hexone bases, lysine and histidine, but was unable to detect them. The significance of the amino-acids has been made clearer by the demonstration that they are the fundamental compounds in the protein molecule. Moreover, improved methods of hydrolysis have placed in our hands the

1 Ascoli: loc. cit.
2 Bergell and Liepman: loc. cit.
5 Mathes: loc. cit.
6 Basso: loc. cit.
means of determining what amino-acids are present in a given material as well as of estimating their quantitative relation. Appreciable differences have already been demonstrated in the amino-acid constitution of various proteins, and it is not improbable that they are intimately associated with differences in physiological activity.

**EXPERIMENTAL DATA.**

Our material has been obtained from the Obstetrical Department of the Johns Hopkins Hospital. All the placentae were normal and at full term. They were received at the laboratory within an hour after their delivery from the uterus, and were kept on ice until the following morning, when they were weighed, examined, and prepared for subsequent analysis.

The umbilical cord and the membranes were first removed. The membranous portion of the chorion, which forms the foetal surface of the placenta, and includes the large blood vessels, was carefully dissected off. Thus, there was left that portion of the chorion which contains the villi, together with a thin layer of decidua which covers the maternal surface of the placenta. Clearly this is the only part of the organ which has to do with the immediate transmission of substances to and from the foetus. Our interest has been to learn what amino-acids enter into the composition of the tissues concerned in the interchange. We have sought, therefore, to eliminate practically everything but the chorionic villi.

The blood was removed as completely as possible. Clots of maternal blood were easily wiped away. After some of the foetal blood was squeezed out by moderate pressure between the hands, the organ was cut into small pieces. These were rinsed in water, then thrown into a towel and wrung out. The process was twice repeated.

The weight of the material was again taken at this stage of the preparation and was found to represent 56 per cent of the original weight of the placenta. Since it is impossible to wring out the material with the same degree of thoroughness each time, there were variations in the different installments. The extremes met with were 37.5 per cent and 62.7 per cent. While not insisting on an absolute figure, it is of interest to note that approximately one-half of the weight of the placenta is accounted for by the foetal blood it contains.
The washed placental substance was next dried in an oven at 100° to 105° C. and after cooling to room temperature, was weighed. The various installments were mixed and finely powdered. This product furnished the material used for our analyses.

There were collected a total of 83 placentae; the sum of their original weights was 47220 grams. From these we secured 3320 grams of dry material which represents 7.03 per cent of the weight of the fresh organs. This ratio remained true, within comparatively narrow limits for all the installments.

### Tabulation of Material Collected

<table>
<thead>
<tr>
<th>INSTALLMENT</th>
<th>NUMBER OF PLACENTÆ</th>
<th>ORIGINAL WEIGHT (grams)</th>
<th>YIELD OF DRY SUBSTANCE (grams)</th>
<th>PERCENTAGE OF DRY SUBSTANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>4</td>
<td>2350</td>
<td>161</td>
<td>6.8</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>930</td>
<td>55</td>
<td>5.9</td>
</tr>
<tr>
<td>III</td>
<td>4</td>
<td>3460</td>
<td>193</td>
<td>5.6</td>
</tr>
<tr>
<td>IV</td>
<td>6</td>
<td>3150</td>
<td>190</td>
<td>6.0</td>
</tr>
<tr>
<td>V</td>
<td>2</td>
<td>1100</td>
<td>85</td>
<td>7.7</td>
</tr>
<tr>
<td>VI</td>
<td>4</td>
<td>2080</td>
<td>173</td>
<td>8.3</td>
</tr>
<tr>
<td>VII</td>
<td>4</td>
<td>2280</td>
<td>197</td>
<td>8.6</td>
</tr>
<tr>
<td>VIII</td>
<td>8</td>
<td>4640</td>
<td>320</td>
<td>6.4</td>
</tr>
<tr>
<td>IX</td>
<td>4</td>
<td>2560</td>
<td>198</td>
<td>7.7</td>
</tr>
<tr>
<td>X</td>
<td>45</td>
<td>24270</td>
<td>1748</td>
<td>7.2</td>
</tr>
</tbody>
</table>

| Total       | 83                  | 47220                   | 3320                          | 7.03                        |

Before proceeding with the analyses, we extracted the material with ether in order to remove fat and other ether-soluble matter. The material was then spread upon large sheets of filter paper and subsequently dried at 105° C.

**Determination of Moisture.** 1.5084 gram substance lost 0.0183 gram when dried to constant weight at 110° C. Therefore, 1.21 per cent of the material used in subsequent hydrolyses was moisture.

**Determination of Ash.** 1.5084 gram substance yielded 0.0992 gram ash. Ash = 6.58 per cent.

**Determination of Nitrogen.** 0.5840 gram substance gave NH₃ = 57.4 cc. \( \frac{m}{20} \) H₂SO₄; N = 13.76 per cent. 0.4842 gram substance gave NH₃ = 47.6 cc. \( \frac{m}{20} \) H₂SO₄; N = 13.77 per cent. Calculated on ash- and moisture-free substance, N = 14.9 per cent.
We have followed the method described by Emil Fischer.\(^1\)

Five hundred grams of the powdered, ether-extracted material were hydrolyzed with boiling hydrochloric acid for twelve hours. This material is equivalent to 461 grams of the substance, free of ash and moisture.

The unhydrolyzed residue weighed 42 grams. Therefore, the weight of the hydrolyzed material was 419 grams.

After removal of the unhydrolyzed material, the filtrate was evaporated \textit{in vacuo}. It was then esterified with three liters of absolute alcohol and saturated with gaseous hydrochloric acid. This liquid was inoculated with a few crystals of glycocoll-ester-hydrochloride and placed in an ice box for forty-eight hours. Subsequent examination failed to reveal any crystallization. Accordingly, the solution was again evaporated \textit{in vacuo}, re-esterified, and allowed to stand another forty-eight hours. This second attempt to isolate glycocoll-ester-hydrochloride was also unsuccessful.

The solution which contained the ester-hydrochlorides was next evaporated \textit{in vacuo} at 40° C. It was treated in the usual way to set the esters free and the esters were extracted with a large quantity of ether. Having stood over night in contact with sodium sulphate, the ethereal extract was evaporated to an appropriate volume and then subjected to fractional distillation. The following fractions were obtained:

<table>
<thead>
<tr>
<th>FRACTION</th>
<th>PRESSURE (mm.)</th>
<th>TEMPERATURE</th>
<th>WEIGHT OF ESTERS (grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>11</td>
<td>Up to 100° C</td>
<td>108.3</td>
</tr>
<tr>
<td>II</td>
<td>0.35</td>
<td>Up to 100° C</td>
<td>38.0</td>
</tr>
<tr>
<td>III</td>
<td>0.15</td>
<td>100° to 190° C</td>
<td>86.2</td>
</tr>
<tr>
<td>Residue</td>
<td></td>
<td></td>
<td>33.4</td>
</tr>
</tbody>
</table>

Proceeding with the treatment of these fractions according to Fischer we have isolated the following amino-acids.

\textit{Glycocoll}. Although unable to isolate this body after primary esterification of the hydrolysed material, we have found it in the first fraction of the distillation-products from which it was isolated as the ester-hydrochloride.

Amount found was 5.75 grams, equivalent to 3.1 grams of free glycocoll.

Amino-Acids in the Placenta

Analysis (Volhard): 0.2575 gram substance required 18.25 cc. \( \tfrac{9}{10} \) AgNO\(_3\)

\[
\begin{align*}
\text{Calculated for} & \quad \text{Found:} \\
\text{Cl} & \quad 25.41 \quad 25.13 \\
\end{align*}
\]

\( d \)-Valine. Amount found was 30.60 grams.

Analysis: 0.2062 gram substance yielded 0.3872 gram CO\(_2\) and 0.1763 gram H\(_2\)O.

\[
\begin{align*}
\text{Calculated for} & \quad \text{Found:} \\
C & \quad 51.24 \quad 51.21 \\
H & \quad 9.47 \quad 9.56 \\
\end{align*}
\]

Specific rotation \((\alpha)_{D}^{20}\) in 20 per cent hydrochloric acid (10 per cent solution) = +25.6° (+0.4°). Theoretical value = +28.8°

\( l \)-Leucine. Amount found was 20.36 grams.

Analysis: 0.1865 gram substance yielded 0.3746 gram CO\(_2\) and 0.1695 gram H\(_2\)O.

\[
\begin{align*}
\text{Calculated for} & \quad \text{Found:} \\
C & \quad 54.92 \quad 54.78 \\
H & \quad 9.99 \quad 10.16 \\
\end{align*}
\]

Specific rotation \((\alpha)_{D}^{20}\) in 20 per cent hydrochloric acid (10 per cent solution) = +14.8° (+0.4°). Theoretical value = +15.9°

Proline. Both racemic and active prolin were isolated. Racemic copper proline: amount found was 1.63 grams. Equivalent in free proline is 0.81 grams.

Water of crystallization of copper salt was determined.

Analysis: 0.5902 gram dried at 108° to a constant weight lost 0.6645 gram H\(_2\)O.

\[
\begin{align*}
\text{Calculated for} & \quad \text{Found:} \\
H\(_2\)O & \quad 10.99 \quad 10.93 \\
\end{align*}
\]

\( l \)-Proline: amount isolated was 10.64 grams.

Analysis: 0.1896 gram substance yielded 0.3475 gram CO\(_2\) and 0.1316 gram H\(_2\)O.

\[
\begin{align*}
\text{Calculated for} & \quad \text{Found:} \\
C & \quad 52.14 \quad 50.6 \\
H & \quad 7.88 \quad 7.76 \\
\end{align*}
\]

The analysis indicates that the \( l \)-proline was impure. Several attempts to purify it were futile. Hence, the amount of pure \( l \)-proline was determined according to the optical rotation of the
various fractions which had been obtained. Thus corrected, the amount of l-proline found was 7.17 grams.

The optical rotation of the analysed substance was determined in a 10 per cent aqueous solution \((\theta \lambda)_d = -57.1^\circ (\equiv 0.2^\circ)\). The theoretical value for pure l-prolin = \(-81.5^\circ\).

**Phenylalanine.** Amount isolated as the hydrochloride was 12.26 grams. Equivalent in free phenylalanine is 10.08 grams.

Analysis (Volhard): 0.3079 gram of the hydrochloride required \(\frac{N}{10}\) AgNO₃.

<table>
<thead>
<tr>
<th>Calculated for (C_9H_7O_3N.HCl):</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl..................................................</td>
<td>17.59</td>
</tr>
<tr>
<td></td>
<td>17.33</td>
</tr>
</tbody>
</table>

Since the material was inactive to polarized light, it must have consisted of the racemic acid.

**Aspartic Acid.** Amount of the racemic acid found was 9.44 grams.

Analysis: 0.2066 gram substance yielded 0.2741 gram CO₂ and 0.0985 gram H₂O.

<table>
<thead>
<tr>
<th>Calculated for (C_4H_7O_4N):</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td>C...............................................</td>
<td>36.07</td>
</tr>
<tr>
<td>H...............................................</td>
<td>5.30</td>
</tr>
</tbody>
</table>

Since the material was inactive to polarized light, it must have consisted of the racemic acid.

**d-Glutaminic Acid.** Amount isolated as the hydrochloride was 15.91 grams. Equivalent in free glutaminic acid is 12.68 grams.

Analysis (Volhard): 0.1874 gram substance required \(\frac{N}{10}\) AgNO₃.

<table>
<thead>
<tr>
<th>Calculated for (C_6H_9O_4NHCl):</th>
<th>Found:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl...............................................</td>
<td>19.21</td>
</tr>
<tr>
<td></td>
<td>19.48</td>
</tr>
</tbody>
</table>

The specific rotation was determined in dilute hydrochloric acid \((\theta \lambda)_d = + 27.5^\circ (\equiv 0.2^\circ)\). Theoretical value = + 30.5^\circ.

**Alanine.** It was impossible to isolate alanine: if present, it occurs in extremely small quantity.

**l-Tyrosine.** We have made a separate hydrolysis with sulphuric acid to determine the amount of tyrosine in the placenta.¹

Two hundred and fifty grams of the powdered ether extracted material was taken. This is equivalent to 230.5 grams ash and moisture-free substance. Non-hydrolyzed material weighed 26.3 grams. Amount of tyrosine found was 3.86 grams.

¹Emil Fischer: loc. cit., p. 88.
**Amino-Acids in the Placenta**

*Analysis:* 0.2092 gram substance yielded 0.4567 gram CO$_2$ and 0.1186 gram H$_2$O.

Calculated for C$_6$H$_8$O$_2$N: Found:

<table>
<thead>
<tr>
<th>Element</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>59.64</td>
<td>59.54</td>
</tr>
<tr>
<td>H</td>
<td>6.12</td>
<td>6.34</td>
</tr>
</tbody>
</table>

The specific rotation was determined in 4 per cent hydrochloric acid (5 per cent solution). ($\alpha$)$_D^{19}$ = $-11.3^\circ$ ($\pm 0.2^\circ$). Theoretical value = $-13.2^\circ$.

**Determination of Hexone Bases.**

The method used was that of Kossel and Kutscher. Fifty grams of the powdered ether-extracted material, equivalent to 46.1 grams ash-and moisture-free substance, were taken.

**Lysine.** Amount isolated as the picrate was 4.10 grams, equivalent to 1.6 grams of free lysine.

*Analysis:* 0.2049 gram substance yielded 0.2333 gram CO$_2$ and 0.0332 gram H$_2$O.

Calculated for C$_6$H$_8$N$_2$O$_2$: Found:

<table>
<thead>
<tr>
<th>Element</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>38.4</td>
<td>38.44</td>
</tr>
<tr>
<td>H</td>
<td>4.53</td>
<td>4.81</td>
</tr>
</tbody>
</table>

**Arginine.** Amount isolated as the mononitrate was 3.25 grams. This was recrystallized from 85 per cent alcohol, but a combustion revealed that the material was still impure. Consequently, it was converted into the methylester hydrochloride.

1.62 grams mononitrate yielded 1.49 grams methyl-ester hydrochloride. Total equivalent in free arginine is 2.00 grams. Melting point of the ester hydrochloride isolated was 193$^\circ$ (uncorr.).

*Analysis* (Volhard): 0.2027 gram required 15.35 cc. $\frac{N}{10}$ AgNO$_3$.

Calculated for C$_7$H$_8$N$_2$O$_2$HCl: Found:

<table>
<thead>
<tr>
<th>Element</th>
<th>Calculated</th>
<th>Found</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cl</td>
<td>27.16</td>
<td>26.85</td>
</tr>
</tbody>
</table>

**Histidine.** Amount of crude substance isolated as the dichloride was 1.66 grams. This material was recrystallized from hydrochloric acid; even then the analysis did not yield the theoretical values. By persistent recrystallization 0.166 grams of crystalline histidine dichloride was isolated from the crude material. Its melting point was 223$^\circ$-225$^\circ$ (uncorr.). Equivalent of free histidine is 0.11 gram.

2*Berichte,* xxxviii, p. 4173, 1905.
Analysis (Volhard): 0.1450 gram substance required 12.55 cc. $\frac{N}{10}$ AgNO$_3$.

**Tryptophane.** The presence of tryptophane was demonstrated as follows: one gram of the crude placental powder was allowed to digest with 10 cc. of a 2 per cent solution of trypsin. The filtered solution gave an intense violet color with bromine water. Control tests with the placental powder and the trypsin solution itself were made simultaneously.

**Ammonia.** The hydrolyzed substance contained 1.28 per cent ammonia (Hart's method).¹

The placental material used for the hydrolyses contained 92.2 per cent of organic matter and approximately one-third of this is accounted for by the products we have isolated. In view of the fact that Fischer's method of hydrolysis fails to give a theoretical yield even when isolation of known mixtures of aminoacids is attempted, one could not expect to recover the aminoacids quantitatively from the placenta. To determine the accuracy of the ester method, Osborne and Jones² who have made such mixtures, have shown that the yield varies between 41 per cent and 82 per cent of the theoretical quantity. Moreover, they have worked under conditions which practically assure complete esterification.

We have not attempted the isolation of cystine, serine, oxyproline and tryptophane; though the presence of the last was demonstrated after tryptic digestion by the bromine reaction. We have made a careful search for alanine but were unable to isolate it. While not inclined to insist absolutely upon its absence, we are confident that, if present at all, extremely small amounts occur in the placenta.

An excess of valine over leucine can hardly be attributed to a peculiarity in the composition of the placenta. Levene and Van Slyke³ have found that the quantity of valine in several proteins is greater than was formerly thought.

Contrary to the opinion of Rielander\textsuperscript{1} lysine and histidine does occur in the placenta, though only a very small quantity of the latter was isolated. Arginine is the most abundant of the hexone bases.

Our hydrolytic products account for about two-fifths of the total nitrogen of the placenta ranked according to the nitrogen they account for, the monamino-acids take the following order: valine, leucine, glutaminic acid, proline, aspartic acid, phenylalanine, tyrosine and glycocoll.

**SUMMARY.**

I. Approximately one-half of the fresh placenta is blood.
II. The dried placental material is about 7 per cent of the gross weight of the organ.
III. The hydrolytic products represent at least 31 per cent of the organic matter.
IV. Placental tissue, free of ash and moisture contains 14.9 per cent of nitrogen.
V. The nitrogen in the isolated products represents 5.6 per cent of the weight of the material used for hydrolysis.
VI. The relative amounts of the amino-acids in the placenta are as follows:
<table>
<thead>
<tr>
<th></th>
<th>Amount in 461 grams placental material, free of ash and moisture</th>
<th>Percentage of placental material</th>
<th>Nitrogen as per cent of placental material</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycocoll</td>
<td>3.10</td>
<td>0.63</td>
<td>0.118</td>
</tr>
<tr>
<td>Alanine</td>
<td>?</td>
<td>?</td>
<td>?</td>
</tr>
<tr>
<td>Valine</td>
<td>30.60</td>
<td>6.64</td>
<td>0.794</td>
</tr>
<tr>
<td>Leucine</td>
<td>20.36</td>
<td>4.42</td>
<td>0.473</td>
</tr>
<tr>
<td>Proline</td>
<td>7.98</td>
<td>1.73</td>
<td>0.228</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>10.08</td>
<td>2.19</td>
<td>0.186</td>
</tr>
<tr>
<td>Glutaminic acid</td>
<td>12.68</td>
<td>2.75</td>
<td>0.262</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>9.44</td>
<td>2.05</td>
<td>0.216</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>7.72</td>
<td>1.68</td>
<td>0.130</td>
</tr>
<tr>
<td>Lysine</td>
<td>16.00</td>
<td>3.46</td>
<td>0.664</td>
</tr>
<tr>
<td>Arginine</td>
<td>20.00</td>
<td>4.33</td>
<td>1.394</td>
</tr>
<tr>
<td>Histidine</td>
<td>1.10</td>
<td>0.24</td>
<td>0.064</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>present</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ammonia</td>
<td>1.28</td>
<td>1.054</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>31.40</td>
<td>5.583</td>
</tr>
</tbody>
</table>

Nitrogen in non-hydrolyzable substance: 0.146

5.729