EXTRACORPOREAL PERFUSION OF THE HUMAN FOETUS, PLACENTA AND FOETO-PLACENTAL UNIT

By
U. Lerner, B. N. Saxena and E. Diczfalusi

ABSTRACT

A system is described for the extracorporeal perfusion of the human foeto-placental unit at midgestation. The same system is used for the perfusion of isolated midgestation foetuses and midgestation or term placentas.

Various gas mixtures were used for the oxygenation of the blood to be perfused, and the pH, pO₂ and pCO₂ values were monitored at frequent intervals in the perfused blood as well as in the various perfusates.

Compared with the values reported for maternal arterial blood, oxygenation of the blood to be perfused with an air + CO₂ mixture (23.1% O₂ + 3.5–3.7% CO₂) yielded a normal pH and almost normal pO₂ and pCO₂ values, whereas oxygenation with carbogen (93.7% O₂ + 6.3% CO₂) resulted in low pH, highly elevated pO₂ and almost normal pCO₂ values.

In all foetal, placental and foeto-placental perfusions conducted at 36°C, the use of blood oxygenated with the air + CO₂ mixture resulted in significantly better pH, pO₂ and pCO₂ values of the perfusates than when blood oxygenated with carbogen was employed. However, the pH, pO₂ and pCO₂ values in the perfusates obtained in the former series of experiments were still outside the range of normal values reported in the literature.

a) Ford Foundation Fellow in Reproductive Endocrinology.
b) Present address: Christian Medical College, Vellore, South India.
Various techniques have been described for the perfusion of the human placenta (e.g. Troen & Gordon 1958; Panigel 1962; Krantz 1962; Varangot et al. 1965; Tojo et al. 1970; Hamrin et al. 1971) and previable foetus (Westin et al. 1958). However, until recently no in vitro methods were available for the perfusion of the complete foeto-placental unit. Previous studies on foeto-placental steroidogenesis were mainly conducted by the use of in situ techniques, introducing tracer amounts of steroids into the amniotic fluid, or into the umbilical circulation at laparotomy, prior to the interruption of gestation (for a review, see Diczfalusy 1969). Other experimental techniques involved the in situ perfusion of midgestation or term placentas (Cassmer 1959; Bolté et al. 1964; Meeker et al. 1971; Jaffe & Ledger 1966; Pion et al. 1966).

These procedures imposed great limitations not only with regard to the amount of radioactive material which could be administered without hazards, but also with respect to the type of labelled compounds which could be used. Thus it was felt to be risky to administer, in such in situ studies, small molecular weight material, such as acetate, pyruvate, glucose, etc., because the radioactive material derived from such precursors is known to be incorporated into a large number of ubiquitously occurring compounds and thus the administration of such precursors would result in a prolonged retention of radioactive material in all maternal tissues.

To overcome the difficulties mentioned above, a method has been developed for the in vitro perfusion of the foeto-placental unit. A preliminary description of the principles involved has been presented elsewhere (Lerner & Diczfalusy 1968; Telegdy et al. 1970; Diczfalusy 1970). Subsequently this technique was applied to the in vitro perfusion of the isolated midgestation placenta (Telegdy et al. 1970b; van Leusden et al. 1971) and isolated midgestation foetus (Mathur et al. 1970; Archer et al. 1971) under experimental conditions in which the pH, pO₂ and pCO₂ values of the perfusates were carefully controlled.

The purpose of the present study was to assess the effect of various gas mixtures upon the pH, pO₂ and pCO₂ values of the perfused blood and perfusates obtained under different experimental conditions.

**MATERIAL AND METHODS**

**Clinical material.** — This was obtained at laparotomy through abdominal hysterotomy from patients who were admitted to the hospital for surgical interruption of gestation for social medical reasons. The period of gestation was between the 15th and 20th weeks. Permission for interruption of gestation was granted upon request of the patient by the Swedish National Board of Health and Welfare under the statute of 1938 as amended in 1946 and 1953.
Following the removal of the products of conception, these were transported in an environment of 36°C to the laboratory without delay; catheters were inserted immediately and perfusion started as soon as possible. Although every effort was made to reduce the time elapsing between the removal of the conceptus and the commencement of the perfusion, this interval still amounted to 10–15 minutes. It is realized that this delay may constitute a significant source of error.

*Perfusion apparatus.* – The apparatus used has been described by *Westin et al.* (1958). It was purchased from Kifa AB, Stockholm, and was slightly modified as indicated in Fig. 1.

*Gas mixtures.* – Two gas mixtures (purchased from Aga AB, Stockholm) were used: a) carbogen (93.5% O₂ + 6.5% CO₂) and b) air containing a controlled amount of oxygen and carbon dioxide (23.1% O₂ + 3.5–3.7% CO₂). Each tube was specially analyzed for the purpose of this study by the analytical laboratory of Aga AB.

*Measurement of pH, pO₂ and pCO₂ of the blood.* – The measurements were carried out in an Acid-Base Analyzer (type PHM 71), using the pO₂ module (type PHA 930) and the pCO₂ module (type PHA 931) according to *Astrup et al.* (1960). The instrument was purchased from Radiometer AS, Copenhagen, Denmark.

*Perfusion of the foeto-placental unit.* – The technique developed in this laboratory is indicated schematically in Fig. 1.

The foetus is placed in an artificial amniotic fluid (indicated in the figure by C), consisting of 5.5% (w/v) glucose in isotonic saline. The temperature of this fluid is kept between 36 and 37°C. The placenta still attached to the foetus is placed carefully into a small, separate container (I) submerged in the artificial amniotic fluid and filled with the same blood that is used for perfusion.

The oxygenator (A) is filled with heparinized, oxygenated, fresh Rh+ O blood diluted with 15% (v/v) isotonic saline. A catheter (No. 1731 or 1732 »Bardic« purchased from Bard-Davol Ltd., Clacton-on-sea, Essex, Great Britain) is inserted into an umbilical artery (B) in the direction of the placenta, and another one (D) in the other artery in the direction of the foetus. The complete foeto-placental unit is then perfused via catheter B. The blood bathing the placenta is continuously replaced by fresh blood from the oxygenator, and a perfusate (called »maternal perfusate«, indicated by E) is collected. Another perfusate (indicated by F) is continuously collected from the foetus. In those studies in which radioactive material is administered, this
is introduced into the oxygenated blood (as indicated by $H$) at a constant rate, using an infusion pump (manufactured by Mr. E. Kcjbo, Stockholm). In this system the perfused material reaches the foetus via the placenta through the umbilical vein; since both umbilical arteries are cannulated, there is no recirculation of labelled material from the foetus to the placenta.

In certain experiments the above system was completed by the introduction of a T-catheter into the umbilical vein (see Diczfalussy 1969, Fig. 1). This arrangement makes it possible to introduce, during ongoing perfusion, labelled material directly into the foetal compartment of the foeto-placental unit.

Using the technique indicated in Fig. 1, the foeto-placental system can be maintained in a fairly good condition for a period of 2–3 hours. It should be noted that no blood pump is employed in any of the perfusions.

**Perfusion of the isolated placenta.** – The conditions used are identical with those indicated above, except that the placenta is perfused via an umbilical artery and the perfusate is collected from the umbilical vein. The placenta is kept in a container filled with blood, which is submerged in the artificial amniotic fluid. The same technique is used for the perfusion of term placentas. The difference in height between the level of the oxygenator (cf. Fig. 1) and
the catheter inserted into the cord is kept at 110 cm when placentas are perfused, whereas in the perfusion of the foeto-placental unit, or of the isolated foetus, this difference is kept at 80 cm.

**Perfusion of the isolated foetus.** – The experimental conditions are the same as those used in the perfusion of the foeto-placental unit, except that the oxygenated blood containing the labelled material reaches the foetus *via* a catheter inserted into the umbilical vein. A perfusate is collected through a catheter inserted into one of the umbilical arteries.

### Results

**Conditions in the oxygenator.** – In order to assess the conditions which prevail in the blood to be perfused during the 2 hours of an average perfusion, four sets of experiments were carried out on 5 different blood samples, as follows. Group No. 1 was oxygenated with carbogen 5.0 litres/min. Carbogen was the gas mixture also in group 2, but with a flow of 0.5 to 0.8 litres/min. The blood samples in groups No. 3 and 4 were oxygenated with air + CO₂; the flow was 5.0 litres/min (group No. 3) and 0.5–0.8 litres/min (group No. 4). Blood samples were removed following oxygenation for 0, 7.5, 15, 30, 60, 90 and 120 minutes, and the pH, pO₂ and pCO₂ values were measured.

An example of the procedure followed is indicated in Tables 1 and 2.

Table 1 indicates the individual pH values found in the oxygenator at various intervals, using different gas mixtures, whereas Table 2 indicates the analysis of variance for the data of Table 1, including pairwise comparisons. It can be seen that the difference in pH values is not significant between groups 1 and 2, but that these two groups differ highly significantly from the other two groups.

The data presented in Figs. 2–5 have been subjected to the same statistical analysis. Only the final results of these analyses are indicated in the figures.

The average pH, pO₂ and pCO₂ values found in the blood oxygenated with various gas mixtures are presented in Fig. 2, whereas the results of the statistical analysis are shown in Table 3.

It can be seen from the data of Fig. 2 and Table 3 that – irrespective of the rate of gas flow – oxygenation with air resulted in significantly higher pH values and in significantly lower pO₂ and pCO₂ values than oxygenation with carbogen. Furthermore, when air was used for oxygenation, a higher pH value was obtained with 0.5–0.8 litres/min than with 5.0 litres/min. However, in absolute terms, the difference was so small that it did not influence significantly the pO₂ and pCO₂ values. Finally, when carbogen was used for oxy-
genation, the higher flow rate resulted in significantly higher pCO$_2$ values without influencing the pH and pO$_2$ figures. It can also be seen that constant values of the parameters studied were obtained only following 30 minutes of oxygenation. Thus it is important to equilibrate the oxygenator at least for 30 minutes prior to the commencement of a perfusion.

**Perfusion of midgestation placentas.** – The data obtained from the analyses of the blood in the oxygenator suggested that oxygenation with 0.5–0.8 litres/min of the air + CO$_2$ mixtures might be optimal. Therefore, in a series of experiments involving the perfusion of 14 midgestation placentas, this type of oxy-

### Table 1.

Values of pH of the blood to be perfused (oxygenator), when various gas mixtures are used for oxygenation.

<table>
<thead>
<tr>
<th>Group No.</th>
<th>Gas mixture</th>
<th>Experiment No.</th>
<th>Time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>Carbogen 5 l/min</td>
<td>1</td>
<td>7.18 7.20 7.26 7.26 7.22 7.22 7.35</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>7.31 7.20 7.20 7.30 7.26 7.24 7.32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3</td>
<td>7.33 7.25 7.25 7.32 7.32 7.27 7.26</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>7.32 7.18 7.18 7.25 7.20 7.20 7.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>7.35 7.24 7.25 7.29 7.27 7.29 7.24</td>
</tr>
<tr>
<td>Mean</td>
<td></td>
<td>1</td>
<td>7.30 7.21 7.23 7.28 7.25 7.24 7.28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2</td>
<td>7.31 7.21 7.24 7.23 7.26 7.25 7.22</td>
</tr>
<tr>
<td>2</td>
<td>Carbogen 0.5–0.8 l/min</td>
<td>3</td>
<td>7.36 7.27 7.31 7.30 7.26 7.23 7.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7.35 7.25 7.20 7.21 7.20 7.19 7.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>7.28 7.23 7.21 7.29 7.28 7.26 7.25</td>
</tr>
<tr>
<td>Mean</td>
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<td></td>
<td></td>
<td>2</td>
<td>7.32 7.30 7.36 7.40 7.40 7.41 7.41</td>
</tr>
<tr>
<td>3</td>
<td>Air + CO$_2$ 5 l/min</td>
<td>3</td>
<td>7.32 7.33 7.34 7.39 7.34 7.36 7.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4</td>
<td>7.34 7.32 7.34 7.37 7.37 7.40 7.43</td>
</tr>
<tr>
<td></td>
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<td>7.32 7.34 7.36 7.38 7.36 7.30 7.36</td>
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<tr>
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<td>1</td>
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<td>7.34 7.35 7.35 7.37 7.39 7.34 7.32</td>
</tr>
<tr>
<td>4</td>
<td>Air + CO$_2$ 0.5–0.8 l/min</td>
<td>3</td>
<td>7.31 7.30 7.33 7.38 7.34 7.38 7.30</td>
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<tr>
<td></td>
<td></td>
<td>4</td>
<td>7.37 7.41 7.44 7.46 7.44 7.44 7.43</td>
</tr>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>7.37 7.42 7.44 7.45 7.40 7.41 7.38</td>
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<tr>
<td>Mean</td>
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<td>7.34 7.36 7.38 7.41 7.40 7.38 7.37</td>
</tr>
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</table>
genation was compared with the previously used technique, consisting of the use of 5.0 litres/min carbogen. The pH, pO₂ and pCO₂ values of the perfusates are indicated in Fig. 3.

It can be seen from the data of Fig. 3 that oxygenation with the air + CO₂ mixture resulted in higher pH and lower pO₂ and pCO₂ values than oxygenation with carbogen. The differences at 0-time shown in the figures – although they appear to be considerable – were statistically not significant, as indicated by the mean values and their standard deviations (pH: 7.09 ± 0.16 vs. 6.96 ± 0.07; pO₂: 42.1 ± 15.9 vs. 70.4 ± 41.9 and pCO₂: 71.7 ± 30.8 vs. 93.5 ± 26.2).

Perfusion of term placentas. – The data of Fig. 3 also include the results obtained following the perfusion of 6 term placentas, using 0.5–0.8 litres/min of the air + CO₂ mixture. Statistical analysis of the results revealed no significant difference between the data obtained following the perfusion of midgestation and term placentas under the experimental conditions.

Table 2.
Analysis of variance of the data of Table 1; pairwise comparisons of selected effects are also presented.

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>Degrees of freedom</th>
<th>Mean square</th>
<th>F</th>
</tr>
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<tr>
<td>Experiments</td>
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<td>0.14592</td>
<td>95.99</td>
</tr>
<tr>
<td>Time</td>
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<td>0.00528</td>
<td>3.47</td>
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<tr>
<td>Subclasses</td>
<td>27</td>
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<td></td>
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<tr>
<td>Subcl. treated alike</td>
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<td>16.42</td>
</tr>
<tr>
<td>Residual (= within subcl.)</td>
<td>112</td>
<td>0.00152</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>139</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Sum</th>
<th>Divisor</th>
<th>Mean square</th>
<th>F-value</th>
<th>P</th>
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<tr>
<td>(1,2) (3,4)</td>
<td>1</td>
<td>-1</td>
<td>+1</td>
<td>+1</td>
<td>7.79</td>
<td>140</td>
<td>0.43346</td>
<td>285.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(1) (3)</td>
<td>1</td>
<td>0</td>
<td>+1</td>
<td>0</td>
<td>3.62</td>
<td>70</td>
<td>0.18721</td>
<td>132.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
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<td>0</td>
<td>0</td>
<td>+1</td>
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<td>70</td>
<td>0.24841</td>
<td>163.4</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(2) (3)</td>
<td>0</td>
<td>-1</td>
<td>+1</td>
<td>0</td>
<td>3.62</td>
<td>70</td>
<td>0.18721</td>
<td>123.2</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>(2) (4)</td>
<td>0</td>
<td>-1</td>
<td>0</td>
<td>+1</td>
<td>4.17</td>
<td>70</td>
<td>0.24841</td>
<td>163.4</td>
<td>&lt; 0.001</td>
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</tbody>
</table>

M. S. res.: 0.00152.
Mean values of pH, pO₂ and pCO₂ in the blood to be perfused following oxygenation with different gas mixtures during 120 minutes. Each point represents the mean of five experiments. \(\Delta-\Delta-\Delta\): carbogen, 5.0 l/min, \(\Delta-\Delta-\Delta\): carbogen, 0.5–0.8 l/min, \(\bigcirc-\bigcirc-\bigcirc\): air + CO₂, 5.0 l/min, \(\bullet-\bullet-\bullet\): air + CO₂, 0.5–0.8 l/min.

**Fig. 2.**

**Table 3.**

Significance of differences between different types of oxygenation of the blood to be perfused.

<table>
<thead>
<tr>
<th>Oxygenator</th>
<th>Parameter</th>
<th>pH</th>
<th>pO₂</th>
<th>pCO₂</th>
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</thead>
<tbody>
<tr>
<td>Groups</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(1,2)(3,4)</td>
<td>0.001</td>
<td>0.001</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>(1) (2)</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.001</td>
<td></td>
</tr>
<tr>
<td>(1) (3)</td>
<td>&lt; 0.001</td>
<td>&gt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>(1) (4)</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td>&lt; 0.001</td>
<td></td>
</tr>
<tr>
<td>(3) (4)</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td>&gt; 0.05</td>
<td></td>
</tr>
</tbody>
</table>

Groups:
1) Carbogen (93.6% O₂ + 6.4% CO₂) 5 l/min.
2) Carbogen (93.6% O₂ + 6.4% CO₂) 0.5–0.8 l/min.
3) Air + CO₂ (Air + 3.6–3.9% CO₂) 5 l/min.
4) Air + CO₂ (Air + 3.6–3.9% CO₂) 0.5–0.8 l/min.
Perfusion of the foeto-placental unit. – Five foeto-placental units were perfused at midgestation, using 5.0 litres/min carbogen for oxygenation, and the results were compared with those obtained following the perfusion of 12 foeto-placental units oxygenated with 0.5–0.8 litres/min of the air + CO₂ mixture. The duration of these experiments was 90 minutes. The results presented in Fig. 4 indicate that oxygenation with air + CO₂ resulted in significantly higher pH and significantly lower pO₂ and pCO₂ values in the foetal perfusate than oxygenation with carbogen. The differences in pH, pO₂ and pCO₂ values at 0-time were not significant (pH: 7.01 ± 0.08 vs. 6.95 ± 0.14; pO₂: 29.3 ± 13.0 vs. 27.0 ± 20.4; pCO₂: 88.4 ± 17.8 vs. 89.3 ± 8.5).

Perfusion of isolated foetuses. – Seven isolated midgestation foetuses were perfused for 120 minutes using 5.0 litres/min carbogen, and 7 other foetuses under conditions using 5.0 litres/min of the air + CO₂ mixture. The results of these experiments are presented in Fig. 5.

The data of Fig. 5 indicate that oxygenation with the air + CO₂ gas mixture resulted in significantly higher pH and significantly lower pO₂ and pCO₂ values in the perfusates than oxygenation with carbogen. The differences in the initial values at 0-time were not significant in any of the measurements (pH: 7.20–7.30 vs. 7.10–7.15; pO₂: 70–90 mm Hg vs. 50–70 mm Hg; pCO₂: 80–90 mm Hg vs. 60–80 mm Hg).

Mean values of pH, pO₂ and pCO₂ in the perfusates obtained from midgestation (n = 7) and term placenta (n = 6), respectively, during perfusion with blood oxygenated with 5.0 l/min of carbogen (open triangles) and 0.5–0.8 l/min of an air + CO₂ mixture (filled circles).

Fig. 3.
Mean values of pH, pO₂ and pCO₂ in the foetal perfusates during the perfusion of the foeto-placental unit with blood oxygenated with 5.0 l/min of carbogen (open triangles, 5 cases) and 0.5-0.8 l/min of an air + CO₂ mixture (filled circles, 12 cases), respectively.

7.04 ± 0.12 vs. 6.97 ± 0.09; pO₂: 32.6 ± 16.4 vs. 24.4 ± 7.8; pCO₂: 88.7 ± 38.9 vs. 116 ± 37.2).

Flow rates under various experimental conditions. – The mean blood flow in the perfusion of midgestation placentas was 5.0 ml/min with a range between 2.3 and 5.7 ml/min. The corresponding figure for term placentas was 3.8 ml/min with a range of 3.2–4.3 ml/min.

In the perfusion of the foeto-placental unit the mean flow was 2.5 ml (range 2.1–2.9 ml/min). The flow was not influenced by the type of oxygenation.

The mean blood flow in the foetal perfusions was 4.3 ml/min (2.7–6.2) when an air + CO₂ mixture was used for oxygenation and 2.6 ml/min (range 1.7–3.8) when carbogen was used. This difference is not significant.

It is realized that the flow rates in our term placental perfusions are much lower than those reported by others (e.g. Krantz et al. 1962; Varangot et al. 1965). It should be emphasized that in all perfusions only one umbilical artery was catherized. Furthermore, in all types of perfusions an open system was used, without a perfusion pump, and the perfused blood was not recirculated.
Mean values of pH, pO₂ and pCO₂ in the perfusates during perfusion of midgestation foetuses with blood oxygenated with 5.0 l/min of carbogen (open triangles) and 5.0 l/min of an air + CO₂ mixture (open circles), respectively. Each point represents the mean of 7 experiments.

**DISCUSSION**

Several investigators have reported on the pH, pO₂ and pCO₂ values of maternal blood and umbilical blood at different stages of gestation. Representative data are indicated in Table 4.

Perusal of the data of Table 4 seem to indicate fairly comparable figures from the 15th week of gestation to term. The values reported by Newman et al. (1967) were obtained by the use of the method of Saling (1962).

In order to compare the normal values indicated in Table 4 with those found in the present study, the mean pH, pO₂ and pCO₂ values obtained in the different types of perfusions are summarized in Table 5.

A comparison of the data of Tables 4 and 5 reveals that – compared to maternal arterial blood – oxygenation of the blood to be perfused with carbogen resulted in low pH, highly elevated pO₂ and almost normal pCO₂ values, whereas oxygenation with the air + CO₂ mixture yielded a normal pH and almost normal pO₂ and pCO₂ values.

When foetuses or foeto-placental units were perfused with blood oxygenated
Table 4.
Average pH, pO₂ and pCO₂ values of maternal and umbilical blood at different stages of gestation as reported in the literature.

<table>
<thead>
<tr>
<th>Period of gestation</th>
<th>Maternal artery</th>
<th>Umbilical vein</th>
<th>Umbilical artery</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pH</td>
<td>pO₂</td>
<td>pCO₂</td>
<td>pH</td>
</tr>
<tr>
<td>Midgestation</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15-20 weeks</td>
<td>(7.35)</td>
<td>—</td>
<td>(37.7)</td>
<td>7.39</td>
</tr>
<tr>
<td>21-28 weeks</td>
<td>7.44</td>
<td>86.6</td>
<td>30.5</td>
<td>7.35</td>
</tr>
<tr>
<td>29-38 weeks</td>
<td>7.45</td>
<td>85.3</td>
<td>30.9</td>
<td>7.37</td>
</tr>
<tr>
<td>39-42 weeks</td>
<td>7.44</td>
<td>84.3</td>
<td>30.1</td>
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<tr>
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a) These measurements refer to maternal venous blood.
b) Values given as per cent saturation.
c) Determinations carried out according to the method of Saling (1962).
with carbogen, the already low pH of the perfusate diminished further, the pO₂ values became elevated and the pCO₂ values remained too high. On the other hand, when air + CO₂ was used for oxygenation in foetal perfusions, the pH of the perfusate increased (although it still remained low); the pO₂ became higher and the pCO₂ values were significantly diminished, although – admittedly – the values still remained relatively high. Oxygenation with air + CO₂ in the perfusion of the complete foeto-placental unit increased the pH of the foetal perfusate, although insufficiently. This treatment resulted in normal pO₂ values and diminished the originally very high pCO₂ levels, which, however, still remained too high.

When midgestation placentas were perfused with blood oxygenated with carbogen, the pH of the perfusate increased, although not sufficiently, and the pO₂ and pCO₂ values remained rather high. When air + CO₂ was employed for oxygenation, the pH of the perfusate increased more than following oxygenation with carbogen, but it remained lower than the normal pH of the

<table>
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<th>Perfusion</th>
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<tr>
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<td>7.20</td>
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a) Mean values obtained at the time of catheterization.
b) Mean values following 15 to 120 minutes of perfusion.
c) Mean values following 15 to 90 minutes of perfusion.
umbilical vein. In addition, slightly elevated pO₂ and pCO₂ values were reached.

No difference was found between midterm and term placentas when perfused with blood oxygenated with air + CO₂. However – in spite of the very low flow rate – the pH, pO₂ and pCO₂ values in the perfusate of term placentas approached the normal values reported for umbilical vein blood at term.

A comparison of the data of Tables 4 and 5 indicates that the use of the air + CO₂ gas mixture for the oxygenation of the blood to be perfused results in experimental conditions which are superior to those obtained when carbogen is used. However, it also follows from the data presented that the former technique still does not yield values which could be considered normal. Further modifications will be required in order to eliminate the initial acidosis present in the blood of foetuses following their removal from the uterine milieu. Also, additional studies will be needed to find out the influence, if any, of this acidosis on various metabolic processes taking place in perfused foetuses.

A review of the literature reveals that most of the authors who perfused midgestation human foetuses employed the technique of Westin et al. (1958), or the slight modifications thereof which were used in previous studies in this laboratory (e.g. Diczfalusy 1969; Greig & MacNaughton 1967; Solomon et al. 1967). In all these experiments the foetuses were perfused with carbogen and at temperatures considerably lower than 36° (mainly 20–25° C).

Extracorporeal perfusion of the human foeto-placental unit at midgestation was reported thus far only from this laboratory. All studies were carried out at 36–37°C. In several of the studies reported, carbogen was used for oxygenation (Telegdy et al. 1970 a–c; van Leusden et al. 1971). However, in a few recent investigations, the air + CO₂ mixture was employed (Mathur et al. 1970; Archer et al. 1971).

A relatively large number of studies have been reported on the extracorporeal perfusion of human placentas; most of these studies deal with term placentas. Among the more important previous techniques are those of Chesley & Alter (1951), in which isolated cotyledons are perfused, and the early studies of Solomon et al. (1954), in which whole term placentas are used. In the methods described by Troen & Gordon (1958) and Goerke et al. (1961) synthetic perfusion media are employed. In the method described by Panigel (1962) two umbilical arteries are catheterized and the placenta is perfused by gravity. Carbogen is used for oxygenation. Varangot et al. (1965) reviewed some of the earlier literature and presented a method in which a synthetic medium is used to wash the placenta, after which blood is used for perfusion. This blood is oxygenated with a carbogen mixture (95%/0 O₂ + 5%/0 CO₂). In none of the studies mentioned above were systematic pH, pO₂ and pCO₂ measurements reported.

The most advanced technique, which probably approaches the physiological conditions more than any of the "retrograde" methods, is that described by
Krantz and coworkers (1962, 1971). In this system, both the maternal and foetal sides are perfused. The maternal side is perfused with blood oxygenated with 1 to 2 litres/min of a gas mixture which contains 20% oxygen and 2–6% CO₂. The experiments include routinely the measurement of maternal and foetal pO₂ values. The pH of the perfusate collected from the umbilical vein was reported to be between 7.2 and 7.3, and the maternal perfusion rate between 550 and 650 ml/min, whereas the flow rate on the foetal side of the placenta varied between 80 and 120 ml/min. This perfusion system was utilized in steroid metabolic studies by Charreau et al. (1968).

During the past 15 years methods have been developed for the extracorporeal perfusion of the human placenta, foetus and foeto-placental unit. These methods offer unique possibilities for the study of the various metabolic interactions between foetus and placenta. Considerable progress has been made in improving the conditions of perfusions. However, further studies are needed in order to approach more closely the so-called »physiological« conditions. It seems particularly important to establish experimental conditions under which the acidosis of the foetus can be eliminated rapidly.

ACKNOWLEDGMENTS

We are indebted to Dr. N. Wiqvist, Department of Women's Diseases, Karolinska sjukhuset, for providing us with clinical material. We are also indebted to Mrs. Berit Fröysa and Miss Ulla Olsson for their participation in these studies.

The expenses of the investigations reported in this paper were defrayed by research grants from the Swedish Medical Research Council, Swedish International Development Authority and the Ford Foundation, New York.

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DISCUSSION

Panigel: Radioangiography, coloured dyes as well as other experimental procedures
clearly show that when you place an entire human placenta in a physiological fluid
bath or in blood, there is almost no circulation of physiological fluid in the inter¬
villous space. All nutrient, respiratory gases dissolved in the »foetal« perfusate of the

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isolated placenta can diffuse to the »maternal« side, but you do not obtain in vitro the dual circulation which normally characterizes placental function in vivo.

I have a few questions about your technique of perfusion. The first is about catheterization only one of the two umbilical arteries at some distance from the placenta. The umbilical arteries have a very reactive muscular wall and the distance between the point of insertion of the catheter and the placenta may have some importance. This has also a great importance for the functioning of the anastomosis between the two umbilical arteries near the place of insertion of the umbilical cord on the placental disc. Only radiological observation during perfusion of the isolated foetal placental unit can show which are the parts of the placenta which are supplied with perfusion fluid.

My second question relates to the insufficiency of the flow rates in your perfusions. What were the foetal placental flows you obtained all along your experiments with this method of catheterizing only one umbilical artery far from the placental insertion of the cord? The placenta normally functions as a heterogeneous circulatory complex organ, and only cineradioangiographic observation could demonstrate the efficiency of perfusion in these artificial conditions.

You refer to my paper from 1962 (Panigil 1962) stating that we have used a gravimetric technique. We used perfusion pumps since 1956, as described in our papers published in French in 1959 (Panigil 1959a,b; Panigil & Mayer 1959; Mayer et al. 1959) and in English in 1960 (Mayer et al.).

Lerner: Where do we insert our catheters? We can dissect the umbilical artery at about 3–4 cm from the placental insertion, we insert our catheter and try to manipulate it as much as possible into the placenta.

Why do we catheterize only one umbilical artery? There are two main reasons why we did it. We didn't want to have recirculation of perfused material to the foetus and we wanted to start the perfusion as soon as possible. It was shown by Westin et al. (1958) that if you catheterize both umbilical arteries on the foetal side, you get a more increased inflow and outflow than if you catheterize only one artery. On the other hand, Krohn et al. (1970) showed that one can perfuse the whole placenta by just perfusing it through one artery, thanks to the anastomosis present in the cotyledons. You have proved that by cineradioangiography the whole placenta would fill up completely. So, we didn't have too much trouble, although we could not check this by electron microscopy.

Concerning your question about the flow rates in the foeto-placental unit, the values are given in the paper. The mean flow was 2.5 ml per minute, the range 2.1–2.9, and the flow was not influenced by the type of oxygenation.

Panigil: It is a very low flow. Normally it is 200 and more.

Lerner: You are referring to term placentas, where the flow is much higher, and we are talking mainly about midterm placentas and midterm foeto-placental units, where the flow is perhaps 10 or 15 times lower. Nevertheless, we are aware of the fact that our flow rates are unphysiologically low.

Cedard: Three years ago we changed the mixture that we use for perfusion, and we now use a mixture very similar to yours, consisting of 60% nitrogen, 5% CO₂ and 35% oxygen. In some cases we can perfuse only one artery instead of two. Sometimes it is possible with one artery to perfuse the whole organ, sometimes, when we have a problem with one umbilical artery, we cannot perfuse the entire organ.
Jaffe: Dr. Lerner, have you had the opportunity in your foetal perfusions, to assess both the functional integrity of the vasculature following perfusion and also the functional integrity of the foetus? There were times in earlier foetal perfusion experiments when the functional integrity of the foetus was somewhat in question.

Secondly, have you had the opportunity as yet to compare either qualitative or quantitative differences with your new perfusion of the foetus as contrasted with earlier perfusions? Are there differences of significance?

Lerner: To your first question, no, we did not. We were only looking at the liver, the adrenal and brain integrity by simple inspection. We have not used the cineradioangiographic method suggested by Dr. Panigel, which would be interesting to try.

Concerning differences in steroid metabolism under these two experimental conditions, I think that Dr. Diczfalusy may wish to make some comments.

Diczfalusy: Data which will be reported by us (Telegdy, Robin & Diczfalusy, to be published) on placental perfusions, using these present conditions with oxygenation with air at 36°C, and both midgestation and term placentas, indicate that there is a much more significant formation of dehydroepiandrosterone from cholesterol than we found in our previous perfusion studies (Telegdy et al. 1970). This would seem to suggest that there is a more important placental steroidogenetic pathway from maternal and foetal cholesterol than we previously supposed. It is, however, still so that we don't get any cholesterol synthesis in the placenta from acetate neither at midgestation (van Leusden et al. 1971) nor at term (van Leusden, Siemerink, Telegdy & Diczfalusy, to be published).

Urquhart: One topic not yet discussed at this meeting is the question of controlling the composition of a recirculating perfusate, bringing thereby circulating perfusate's composition under control with the inclusion of some kind of dialysis unit in the circuit. It strikes me when listening to you, that Nature in effect provides us with this in the form of the implanted placenta. I just wonder what your thoughts would be about removing the foetus and using the foetal side of the implanted placenta through which to recirculate the perfusate from some outside, in vitro organ and thus using the mother as a metabolic support system for the perfused organ. It is a sort of variant of our pilot organ manoeuvre.

Lerner: I would say that the idea is marvellous. The only problem is the practical feasibility. Perhaps it would be a possibility to combine Dr. Krantz's system with the entire foeto-placental unit, or as Baird et al. (1971) did, just perfuse the whole uterus.

Rocmans: One comment and three short questions: I think 5 l of gas per minute in the oxygenator is very toxic for the red blood cells in relation to the blood flow you have (e.g. 95% O2 - 5% CO2 flow rate during extracorporeal cardiopulmonary pass for thoracic surgery: 4 litres/litre blood flow rate). I am surprised too by the really low blood flow rate and I think it would be interesting, as a general rule, to express the flow rate per gram of tissue and, in your system, for placenta and foetus; do you have any data expressed in this way? Secundo, did you measure, besides the pCO2, the bicarbonate concentration in the medium and, tertio, did you correlate the changes in pCO2, pO2 and pH with changes in haematocrit?

Lerner: It would perhaps be more interesting to express the blood flow in grams per minute, but we did not. One must keep in mind that these foetuses are 16–19 weeks of
age, weighing around 150 g; in the case of the foeto-placental unit, the whole system would weigh no more than some 250 g altogether.

We did not measure the bicarbonate concentration and we did not correlate haematocrit to $pO_2$, $pCO_2$ or pH values.

Rudolph: I thought I might bring this question of flow into context in relation to measurements in normal foetuses. We have made extensive studies of cardiac output and umbilical blood flow in lamb foetuses in utero. In the lamb, the cardiac output is about 450 ml per kg foetal weight per minute with an umbilical-placental flow of about 200–220 ml per kg of foetal weight per minute. We have made some measurements in human foetuses in midgestation while they were still attached to the placenta during hysterectomy. There the measurements were not very different from those of the lamb, so, when you report a flow of 2–3 ml/min in a 150 gram foetus, this is about one fifteenth or 6 per cent of normal flow.

Lerner: I was not aware of your results. There is a recent review by Walsh & Lind (1970) in which they have some data on term human foetuses. The umbilical blood flow was around 150 ml per minute per kg weight. They state in this paper that at midgestation the flow would be much lower. It is increasing gradually with the development of the foetus. When you say cardiac output, one has to realize that not all the blood goes to the placenta, only 50–60 per cent. Now, trying to put together those two figures and extrapolating from the term foetus to midgestation, which may or may not be correct, one would assume that you would have an umbilical flow around 15 ml per 100 g weight. This would correspond more or less to the figures given by Westin in 1958, but with both umbilical arteries catherized. We realize that the values we got are much lower, and we would like to know how to improve them.

Rudolph: I am not aware of the measurements Walsh & Lind obtained in the full term foetus. In our studies there was no question about the fact that at midterm, in the lamb at least, the proportion of cardiac output distributed to the placenta is actually higher than at term. I would suspect, from the measurements we have in the human foetus, that this is probably true in the human as well.

Gurpide: During in vivo experiments with pregnant sheep, we noted that the clearance of labelled progesterone and uridine by the foetus changed drastically when the foetus began to deteriorate, as judged by pH measurements. Fast metabolising compounds could be used to monitor the foetal condition during perfusion. Have you tried anything similar?

Hubinont: Is this a living foetus? Did you have any parameters of heart activity or cord blood flow in these experiments – because it might be either a passive perfusion or there might be an activity on the part of the foetus, and there is a question to what extent one type of perfusion contributes to the other one.

Lerner: Answering Dr. Gurpide: No, we didn’t have any special parameters to assess the correct function of the foetus, just the pH and $pO_2$ and $pCO_2$ values, and, of course, steroid biogenesis.

Professor Hubinont asked how we could know that the foetus was alive. Just by the heart beat; we measured this in at least 10 or 12 perfusions before we started, but just visually. I would like to add, however, that we found it virtually impossible to perfuse the foetus with this technique once the foetal heart stopped functioning. In some foetal perfusions the umbilical vein pressure was around 30–32 mmHg. However, we did not measure this consistently in all experiments.

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Van Bogaert: I want to tell you, Dr. Lerner, that I know from Dr. Krantz that he has tried to keep a midgestation foetus alive. I don't know exactly by what criteria he followed the fact that it was alive, but anyway, he told me that it was for six hours. So this may be a very interesting contribution.

Lerner: I think this is a very interesting comment, which will even answer part of Dr. Urquhart's question.

Jaffe: Just a suggestion: You can easily monitor foetal cardiac rate using a Doppler monitor which you could put on the outside of your perfusion apparatus. You can constantly record the signals from the monitor as well, without even going to the trouble of electrodes.

Hubinont: Concerning this question of pH: at the moment you started your perfusion, you measured the pH. It was 7.03. After perfusing you got to the value of 7.92, if I remember well, but you never get to what is considered as normal pH values in the umbilical vein for this type of pregnancy, midterm pregnancy. What is your explanation for this? Is it a proof of a metabolic acidosis apart from the gaseous acidosis indicating that some tissue damage is going on?

Lerner: The foetuses are removed in the operation theatre of the Department of Obstetrics and Gynecology, which is some 300 metres from our laboratory. Although it takes a short time, four minutes, to run from the operation theatre to the laboratory, nevertheless, before the catheters are inserted and perfusion is started, generally some 14-15 minutes have elapsed. This time may be enough to develop foetal acidosis.

Levitz: I collaborated with Dr. Diczfalusy, using the original Westin foetal perfusion set-up. I recall an occasional passing of meconium by the foetus. Do you think that ultraviolet examination of the »amniotic fluid« could provide an index of foetal distress during perfusion?

Lerner: Dr. Levitz, in the last perfusion we were very fortunate, because we got practically the whole egg, so the amniotic fluid looked absolutely clear, normal. Of course, one would have to assess it by UV, but there could not be seen any signs of foetal distress or anoxia, or even hypoxia.

Hubinont: I would like to settle this question by saying, as an obstetrician, that when the baby is out of the uterus, if there is meconium, it does not signify at all that there is foetal distress. I think this is an important point to make.

References:
