

DONALD H. BARRON\*  
GIACOMO MESCHIA\*\*

Laboratory of Physiology, Yale University  
School of Medicine

### THE CARBON DIOXIDE CONCENTRATION GRADIENT BETWEEN THE FETAL AND MATERNAL BLOODS OF SHEEP AND GOATS†

The observations to be presented here were made in an effort to gain a wider understanding of the circumstances that influence or modify the exchange of the respiratory gases between the fetal and maternal bloods in the placenta through a study of the exchange of carbon dioxide between fetus and mother across the five-layered syndesmochorial placenta of the sheep and goat. We sought (i) to determine the concentrations of the physically dissolved  $\text{CO}_2$  of the plasma bicarbonate and of hydrogen ions that exist in the fetal and maternal bloods in the steady state and (ii) to gain some knowledge of the manner in which these concentrations in fetal plasma respond to variations in the individual levels of the corresponding constituents of the maternal plasma. Our hopes were not fully realized, for a steady state across the placenta is not easily maintained in circumstances that permit the fetal blood to be sampled; nevertheless, the data we have obtained do permit some inferences to be drawn about the steady state equilibrium as well as the manner in which a fall in the dissolved  $\text{CO}_2$  and bicarbonate of the maternal plasma affects the fetal acid-base balance.

#### MATERIALS AND METHODS

The individual sheep and goats‡ selected for this study were brought into the laboratory four or five days before the experiment and kept off feed for the 48 hours preceding it. In preparation for the necessary laparotomy the animals were given either 1.5 to 2 cc. of Spinocain subdurally or intravenous isotonic pentothal sodium

---

\* Professor of Physiology.

\*\* Toscanini Fellow.

† Aided by a grant from the Division of Research Grants and Fellowships, National Institutes of Health, U. S. Public Health Service.

‡ The sheep and goats on which these observations were made were good grade animals. The sheep, Shropshires and Hampshires, were kept as a part of their flock by Dr. and Mrs. Loeb on their farm in Bethany, just outside of New Haven. There they had the run of a good pasture with a ration of hay and grain in the winter months. The goats, kept under similar conditions, were purchased from Mrs. Rudolph Gode of Cheshire. The Loeb's and Mrs. Gode provided us with the breeding dates of the ewes and does on which the estimations of fetal age are based; without their interest and co-operation our study would not have been possible.

*Received for publication April 25, 1956.*

(c. 0.5 cc. per kilo of body weight of a solution made by dissolving one gram in 30 cc. distilled water). The animal was then quickly placed on its back or side and the uterus exposed through an incision that separated all of the abdominal layers at once. A small vein draining a cotyledon of the central portion of the uterine horn containing the fetus was selected and from it, without dissection or handling, a 2 to 5 cc. sample of blood was drawn under oil into a syringe whose walls had been moistened with a neutral heparin-fluoride solution to prevent clotting and glycolysis. After this sample had been drawn, the uterus was opened by a single incision at right angles to the long axis of the horn and the umbilical cord delivered with a minimum of manipulation and handling. Samples of blood—2 to 5 cc.—were then similarly drawn from the umbilical vein, the umbilical artery, and the uterine artery in that order. They were drawn in rapid succession and with the minimum of disturbance to the uterus and the fetus.

In the earlier experiments the syringes were capped and put into a beaker containing melting ice and water. Following the removal of samples for the determination of the oxygen content and capacity, the syringes were transferred to a shallow mercury bath until their contents came to room temperature. The pH of the whole blood was determined by means of a glass electrode and a Beckman meter standardized with a buffer of known hydrogen ion concentration. The results were corrected to 38° C. by means of Rosenthal's<sup>10</sup> equation:

$$\text{Blood pH}_{38} = \text{pH}_t - 0.0147 (38-t) \quad (1)$$

where  $t$  is the temperature at which the pH of the blood is read. We determined by experiment that this correction factor is applicable to the bloods of sheep and goats.

The remainder of the sample was transferred under heavy paraffin oil into a centrifuge tube closed with a one-hole rubber stopper that was in turn closed with a glass rod after all but a thin layer of the oil had been displaced from the tube by the blood. The cells were separated from the plasma by centrifugation at room temperature, i.e., the temperature at which the pH was measured.

In later experiments a portion of the blood, immediately it was drawn, was transferred under oil into centrifuge tubes that had been suspended in a bath at 38° C. The separation of the cells and plasma, as well as the subsequent measurement of the plasma pH, was carried out at 38° C.; the centrifuge and the pH meter were enclosed in cabinets whose air temperature was held at that level thermostatically. The results of the two procedures were similar. The carbon dioxide content of the plasma was determined, in all cases, by Van Slyke's manometric method on 0.5 cc. samples.

The pH and the total CO<sub>2</sub> as determined were used to calculate the concentration of the physically dissolved CO<sub>2</sub> of the plasma by means of the Henderson-Hasselbalch equation:

$$\text{pH} = 6.10 + \log \frac{\text{Total CO}_2 - \text{Physically dissolved CO}_2}{\text{Physically dissolved CO}_2} \quad (2)$$

The solubility coefficient of CO<sub>2</sub> in plasma is 0.0301 millimols per liter for each millimeter Hg of CO<sub>2</sub> pressure.<sup>12</sup> Accordingly, the partial pressure of carbon dioxide with which the physically dissolved CO<sub>2</sub> would be in equilibrium may be obtained by dividing the concentration of the latter by 0.0301. In the adult animal, where the exchange of the respiratory gases in the lungs takes place between a liquid and a gaseous phase, knowledge of the partial pressure of the gases with which the blood is in equilibrium is of obvious importance and the concentrations of the physically dissolved CO<sub>2</sub> are

usually expressed as gas pressures. As the exchange of the respiratory gases across the placenta takes place between two aqueous phases the movement of the physically dissolved  $\text{CO}_2$  from the fetal to the maternal blood depends upon the gradient of its concentration across the barrier. For this reason we have expressed the concentration of the physically held  $\text{CO}_2$  as such, though in earlier publications we referred to the pressures rather than concentrations.

## RESULTS

In all 15 sheep and 3 goats were sacrificed in these experiments; their fetuses ranged from 50 to 144 days of age as reckoned from the date of insemination. Two ewes bore twins. The data obtained by the analysis of the blood samples are arranged in Table 1. The difference in the concentration of the physically dissolved  $\text{CO}_2$  on the two sides of the placental barrier has been estimated by subtracting the value for the concentration in the uterine artery from that of the umbilical vein and correspondingly the concentration in the uterine vein from that in the umbilical artery, for within the placenta of the sheep<sup>1</sup>—and apparently in the goat as well (unpublished)—the fetal and maternal bloods flow in opposite directions in more or less parallel cords of capillaries. As a consequence of this arrangement the maternal blood leaving the placenta via the uterine vein is opposed to the fetal blood entering via the umbilical artery, whereas the fetal blood leaving by the umbilical vein is opposed to maternal blood entering through the uterine artery.

In every case the concentration of the physically dissolved  $\text{CO}_2$  was higher in the fetal plasma than it was in the plasma of the opposing maternal vessel, by amounts ranging from 0.1 to 0.8 millimols per liter. The more commonly occurring difference was of the order of 0.2 millimols per liter with the tendency for the higher values to occur in the young fetuses. However, the number of observations is too limited to be conclusive in this respect.

The bicarbonate content of the fetal plasma is higher than that of the maternal in all cases but one—sheep 144 days, the last entry in Table 1—and in that case the difference is less than a millimol per liter of plasma. In the most extreme case the fetal bicarbonate concentration was 7-9 millimols per liter higher than that of the maternal plasma. The fetal plasma pH was lower than the maternal in the majority of the cases.

## DISCUSSION

The data presented in Table 1 indicate that, despite wide variations in the differences in the bicarbonate and hydrogen ion concentrations of the fetal and maternal plasmas, the difference in the concentrations of physically dis-

TABLE 1

| <i>Animal</i> | <i>Fetal age<br/>Days</i> | <i>Vessel</i> | <i>pH</i> | <i>Physically dissolved</i>      |                                | <i>Difference in physically dissolved CO<sub>2</sub></i> |                          |
|---------------|---------------------------|---------------|-----------|----------------------------------|--------------------------------|--|--------------------------|
|               |                           |               |           | <i>BHCO<sub>2</sub><br/>mM/l</i> | <i>CO<sub>2</sub><br/>mM/l</i> | <i>Umb.V.-<br/>Ut.A.</i>                                 | <i>Umb.A.-<br/>Ut.V.</i> |
| Sheep         | 50                        | Umb. A.       | 7.16      |                                  |                                | 0.2  |                          |
|               |                           | Umb. V.       | 7.43      | 24.4                             | 1.1                            |  |                          |
|               |                           | Ut. A.        | 7.46      | 21.3                             | 0.9                            |  |                          |
|               |                           | Ut. V.        | 7.41      | 21.8                             | 1.1                            |  |                          |
| Sheep         | 56                        | Umb. A.       | 7.17      | 23.5                             | 2.0                            | 0.4  | 0.8                      |
|               |                           | Umb. V.       | 7.33      | 23.4                             | 1.4                            |  |                          |
|               |                           | Ut.A.         | 7.35      | 17.2                             | 1.0                            |  |                          |
|               |                           | Ut. V.        | 7.30      | 18.5                             | 1.2                            |  |                          |
| Sheep         | 65                        | Umb. A.       | 7.28      | 24.1                             | 1.6                            |  | 0.5                      |
|               |                           | Umb. V.       | ...       | ...                              | ...                            |  |                          |
|               |                           | Ut. A.        | 7.27      | 13.3                             | 0.9                            |  |                          |
|               |                           | Ut. V.        | 7.22      | 14.2                             | 1.1                            |  |                          |
| Sheep         | 81                        | Umb. A.       | 7.36      | 20.8                             | 1.1                            | 0.4  | 0.3                      |
|               |                           | Umb. V.       | 7.38      | 20.1                             | 1.1                            |  |                          |
|               |                           | Ut. A.        | 7.40      | 13.7                             | 0.7                            |  |                          |
|               |                           | Ut. V.        | 7.37      | 14.1                             | 0.8                            |  |                          |
| Sheep         | 92                        | Umb. A.       | 7.32      | 23.8                             | 1.4                            |  | 0.5                      |
|               |                           | Umb. V.       | 7.34      | 21.3                             | 1.2                            |  |                          |
|               |                           | Ut. A.        | ...       | ...                              | ...                            |  |                          |
|               |                           | Ut. V.        | 7.34      | 15.2                             | 0.9                            |  |                          |
| Sheep         | 120                       | Umb. A.       | 7.37      | 25.7                             | 1.4                            | 0.4  | 0.3                      |
|               |                           | Umb. V.       | 7.40      | 23.9                             | 1.2                            |  |                          |
|               |                           | Ut. A.        | 7.44      | 17.8                             | 0.8                            |  |                          |
|               |                           | Ut. V.        | 7.38      | 20.4                             | 1.1                            |  |                          |
| Sheep         | 126<br>1st twin           | Umb. A.       | 7.27      | 22.4                             | 1.5                            | 0.35   | 0.35                     |
|               |                           | Umb. V.       | 7.31      | 21.5                             | 1.3                            |  |                          |
|               |                           | Ut. A.        | 7.40      | 19.0                             | 0.9                            |  |                          |
|               |                           | Ut. V.        | 7.33      | 19.6                             | 1.2                            |  |                          |
|               | 2nd twin                  | Umb. A.       | 7.21      | 21.3                             | 1.7                            | 0.3  | 0.2                      |
|               |                           | Umb. V.       | 7.27      | 20.0                             | 1.3                            |  |                          |
|               |                           | Ut. A.        | 7.38      | 19.8                             | 1.0                            |  |                          |
|               |                           | Ut. A.        | 7.25      | 20.8                             | 1.5                            |  |                          |

TABLE 1 (continued)

| <i>Animal</i> | <i>Fetal age<br/>Days</i> | <i>Vessel</i> | <i>pH</i> | <i>BHCO<sub>2</sub><br/>mM/l</i> | <i>Physically<br/>dissolved<br/>CO<sub>2</sub><br/>mM/l</i> | <i>Difference in<br/>physically<br/>dissolved CO<sub>2</sub></i> |                          |
|---------------|---------------------------|---------------|-----------|----------------------------------|---|--|--------------------------|
|               |                           |               |           |                                  |   | <i>Umb.V.-<br/>Ut.A.</i>   | <i>Umb.A.-<br/>Ut.V.</i> |
| Goat          | 133                       | Umb. A.       | 7.37      | 31.6                             | 1.7   | 0.3  | 0.3                      |
|               |                           | Umb. V.       | 7.39      | 29.3                             | 1.5   |  |                          |
|               |                           | Ut. A.        | 7.41      | 23.6                             | 1.2   |  |                          |
|               |                           | Ut. V.        | 7.35      | 24.9                             | 1.4   |  |                          |
| Sheep         | 136                       | Umb. A.       | 7.30      | 16.5                             | 1.0   | 0.2  | 0.2                      |
|               |                           | Umb. V.       | 7.34      | 15.5                             | 0.9   |  |                          |
|               |                           | Ut. A.        | 7.43      | 15.2                             | 0.7   |  |                          |
|               |                           | Ut. V.        | 7.38      | 15.4                             | 0.8   |  |                          |
| Sheep         | 136                       | Umb. A.       | 7.35      | 17.0                             | 0.95  | 0.1  | 0.15                     |
|               |                           | Umb. V.       | 7.42      | 16.4                             | 0.8   |  |                          |
|               |                           | Ut. A.        | 7.43      | 14.4                             | 0.7   |  |                          |
|               |                           | Ut. A.        | 7.39      | 15.5                             | 0.8   |  |                          |
| Sheep         | 137<br>1st twin           | Umb. A.       | 7.44      | 20.3                             | 0.9   | 0.2  | 0.2                      |
|               |                           | Umb. V.       | 7.46      | 19.1                             | 0.8   |  |                          |
|               |                           | Ut. A.        | 7.39      | 11.2                             | 0.6   |  |                          |
|               |                           | Ut. V.        | 7.36      | 12.7                             | 0.7   |  |                          |
|               | 2nd twin                  | Umb. A.       | 7.39      | 20.6                             | 1.05  | 0.3  | 0.25                     |
|               |                           | Umb. V.       | 7.42      | 18.0                             | 0.9   |  |                          |
|               |                           | Ut. A.        | 7.39      | 11.7                             | 0.6   |  |                          |
|               |                           | Ut. V.        | 7.32      | 14.0                             | 0.8   |  |                          |
| Goat          | 137                       | Umb. A.       | 7.32      | 30.2                             | 1.8   | 0.2  | 0.2                      |
|               |                           | Umb. V.       | 7.37      | 29.2                             | 1.55  |  |                          |
|               |                           | Ut. A.        | 7.42      | 28.1                             | 1.35  |  |                          |
|               |                           | Ut. V.        | 7.37      | 29.8                             | 1.6   |  |                          |
| Sheep         | 137                       | Umb. A.       | 7.47      | 29.3                             | 1.25  | 0.2  | 0.25                     |
|               |                           | Umb. V.       | 7.49      | 27.7                             | 1.1   |  |                          |
|               |                           | Ut. A.        | 7.45      | 20.8                             | 0.9   |  |                          |
|               |                           | Ut. V.        | 7.43      | 22.3                             | 1.0   |  |                          |
| Sheep         | 138                       | Umb. A.       | 7.32      | 21.4                             | 1.3   | 0.25   | 0.3                      |
|               |                           | Umb. V.       | 7.38      | 19.4                             | 1.0   |  |                          |
|               |                           | Ut. A.        | 7.39      | 14.4                             | 0.75  |  |                          |
|               |                           | Ut. V.        | 7.34      | 17.4                             | 1.0   |  |                          |

TABLE 1 (continued)

| <i>Animal</i> | <i>Fetal age<br/>Days</i> | <i>Vessel</i> | <i>pH</i> | <i>BHCO<sub>3</sub><br/>mM/l</i> | <i>Physically<br/>dissolved<br/>CO<sub>2</sub><br/>mM/l</i> | <i>Difference in<br/>physically<br/>dissolved CO<sub>2</sub></i> |                          |
|---------------|---------------------------|---------------|-----------|----------------------------------|---|--|--------------------------|
|               |                           |               |           |                                  |   | <i>Umb.V.-<br/>Ut.A.</i>   | <i>Umb.A.-<br/>Ut.V.</i> |
| Goat          | 140                       | Umb. A.       | 7.33      | 25.8                             | 1.5   | 0.5  | 0.2                      |
|               |                           | Umb. V.       | 7.38      | 24.3                             | 1.3   |  |                          |
|               |                           | Ut. A.        | 7.44      | 18.4                             | 0.8   |  |                          |
|               |                           | Ut. V.        | 7.32      | 21.9                             | 1.3   |  |                          |
| Sheep         | 142                       | Umb. A.       | 7.30      | 23.7                             | 1.5   | 0.1  | 0.2                      |
|               |                           | Umb. V.       | 7.35      | 22.0                             | 1.2   |  |                          |
|               |                           | Ut. A.        | 7.38      | 20.9                             | 1.1   |  |                          |
|               |                           | Ut. V.        | 7.35      | 23.0                             | 1.3   |  |                          |
| Sheep         | 144                       | Umb. A.       | 7.36      | 26.9                             | 1.5   | 0.1  |                          |
|               |                           | Umb. V.       | 7.40      | 26.4                             | 1.3   |  |                          |
|               |                           | Ut. A.        | 7.45      | 21.0                             | 1.2   |  |                          |
|               |                           | Ut. V.        |           |                                  |   |  |                          |
| Sheep         | 144                       | Umb. A.       | 7.39      | 25.9                             | 1.3   | 0.15   | 0.1                      |
|               |                           | Umb. V.       | 7.43      | 24.5                             | 1.15  |  |                          |
|               |                           | Ut. A.        | 7.51      | 25.1                             | 1.0   |  |                          |
|               |                           | Ut. V.        | 7.45      | 26.6                             | 1.2   |  |                          |

solved CO<sub>2</sub> on the two sides of the placental barrier is fairly constant if we consider only those fetuses 120 days or older; the average difference in concentration at the arterial ends (Umb.V.-Ut.A.) of the parallel capillary cords in this group (120-140 days) is 0.25 millimols per liter of plasma. The average difference at the venous end (Umb.A.-Ut.V.) is 0.23 millimols. In other words, it appears one may take the value 0.24 millimols per liter as a representative figure for the difference in the concentration of the physically dissolved CO<sub>2</sub> along the capillaries for fetuses of this group.

Results of this same order have been reported in the few cases in which similar studies were made in the cow and the goat, both animals with a syndesmochorial placenta. For example, Huggett,<sup>5</sup> the pioneer in the study of the placental exchange of the respiratory gases, found the carbon dioxide tension in the umbilical artery to be 18 mm. higher than that of the uterine vein, a pressure difference that corresponds to a difference in the concentration of physically dissolved CO<sub>2</sub> of 0.54 millimols per liter of plasma.

Keys in a study also made on goats, and in which he used a modification of Krogh's bubble method, found the CO<sub>2</sub> tension in the umbilical vein to exceed that of the uterine vein by 2 and 6 mm. in two cases corresponding to differences in the concentration of the physically dissolved CO<sub>2</sub> of *circa* 0.06 and 0.18 millimols per liter of plasma respectively. These observations were made before the pattern of counterflow in the placenta had been widely recognized, so the data are of interest though of somewhat limited significance. Keys<sup>8</sup> estimated the CO<sub>2</sub> pressures in the fetal and maternal bloods of goats in four other cases but unfortunately for our purpose the particular vessel from which the samples were drawn is not mentioned. More useful for comparative purposes are the data of Roos and Romijin<sup>9</sup> on the cow; this animal like the sheep and goat has a five-layered syndesmochorial placenta. Roos and Romijin estimated the difference in CO<sub>2</sub> pressure between the fetal and maternal bloods by referring the CO<sub>2</sub> content of the plasmas of samples drawn from each of the four placental vessels to the appropriate carbon dioxide dissociation curve prepared with larger samples of the fetal and maternal bloods. The four cows they studied were in the final third of the gestation period; the difference in CO<sub>2</sub> pressures at the arterial end of the placenta (Umb.V.-Ut.A.) they found to range between 3.5 and 32 mm. Hg and at the venous end (Umb.A.-Ut.V.) between 4 and 23 mm. Hg. These differences in pressure correspond to differences in the concentration of physically dissolved carbon dioxide of 0.1-1.0 and 0.1-0.7 millimols per liter at the arterial and venous ends, respectively, of the placental circulation.

In all of the cases described above, as well as in our own, the assumption was made that the concentrations of physically dissolved carbon dioxide in the four major vessels, uterine artery, uterine vein, umbilical artery, and umbilical vein represent the concentrations that exist at the corresponding ends of the capillaries to and from which they lead; it was further assumed that all of the blood flowing in these major vessels passes through capillaries involved in the exchange of the respiratory gases. However, the possibility must be considered that the difference in the concentrations of CO<sub>2</sub> between the bloods of the larger vessels may be due to the fact that the umbilical and uterine veins collect some blood that was not involved in the exchange of the respiratory gases across the placental barrier. One might assume, for example, that the concentrations of the physically dissolved carbon dioxide come into equilibrium at equality in the fraction of the fetal and maternal bloods that are involved in the respiratory exchange, but not in the remaining portion entering and leaving the uterine and umbilical veins respectively, and that at equality in the physically dissolved CO<sub>2</sub> the concentrations of

the total  $\text{CO}_2$  ( $\text{CO}_2$ ) of the maternal and fetal bloods are approximately equal. In that circumstance the fraction ( $f$ ) of the umbilical flow involved in the respiratory exchange and coming into equilibrium with the maternal arterial blood should be:

$$f = \frac{(\text{CO}_2)_{\text{Umb.A.}} - (\text{CO}_2)_{\text{Umb.V.}}}{(\text{CO}_2)_{\text{Umb.A.}} - (\text{CO}_2)_{\text{Ut.A.}}} \quad (3)$$

If the difference between the physically dissolved  $\text{CO}_2$  in the umbilical vein (Umb.V.) and uterine artery (Ut.A.) is 0.24 mM, the difference in total  $\text{CO}_2$  between the two vessels should be about 1.5 mM in the steady state, as determined by reference to carbon dioxide dissociation curves of fetal and maternal bloods, i.e.:

$$(\text{CO}_2)_{\text{Ut.A.}} = (\text{CO}_2)_{\text{Umb.V.}} + 1.5 \quad (4)$$

Assuming an A-V difference of about 1.5 mM as representative of normal conditions of uterine blood flow:

$$(\text{CO}_2)_{\text{Umb.A.}} = (\text{CO}_2)_{\text{Umb.V.}} + 1.5 \quad (5)$$

In these circumstances equation 3 becomes:

$$f = \frac{(\text{CO}_2)_{\text{Umb.V.}} + 1.5 - (\text{CO}_2)_{\text{Umb.V.}}}{(\text{CO}_2)_{\text{Umb.V.}} + 1.5 - (\text{CO}_2)_{\text{Umb.V.}}} = 0.5 \quad (6)$$

Thus, to account for our results on the basis of the above assumption would require that more than 50 per cent of the uterine blood flow be shunted through channels not involved in the exchange of the respiratory gases. Such a possibility appears to be excluded by inspection, for the organization of the vascular bed of the uterus does not appear to include a by-pass of that magnitude.<sup>1</sup>

On the basis of the above considerations we have accepted 0.24 millimols per liter as representative of the difference in the concentration of the physically dissolved  $\text{CO}_2$  in the plasmas of the maternal and fetal placental capillaries of the sheep during the last month of the gestation period and have used this representative value in an effort to learn something about the physical characteristics of the placental barrier as well as the concentrations of the bicarbonate and hydrogen ion that might be representative of the normal conditions *in utero*.

The difference in concentration of the physically dissolved  $\text{CO}_2$  on the two sides of the placenta provoked the question: in regard to the restriction it places upon the diffusion of carbon dioxide and oxygen does the placenta act as though it were a simple layer of water separating the two plasmas?



On that assumption, i.e., that the placental barrier may be regarded as a layer of water, the quantity of gas moving from the fetal to the maternal plasma, or vice versa, may be expressed according to Fick's law, as follows:

$$Q = \frac{-k A \Delta C}{D} \quad (7)$$

where  $Q$  equals the quantity of gas in millimols transferred per unit time,  $k$  the diffusion constant,  $A$  the area across which diffusion takes place,  $D$ , the thickness of the separating membrane, and  $\Delta C$ , the difference in concentration across the membrane. The R.Q. of the sheep fetus at term is about one (unpublished data). Accordingly, the quantity of oxygen crossing the placental barrier per unit time is equal to that of the carbon dioxide moving in the opposite direction and it follows that:

$$Q_{O_2} = Q_{CO_2} \quad (8)$$

and further that

$$\frac{k_{O_2} A \Delta C_{O_2}}{D} = \frac{k_{CO_2} A \Delta C_{CO_2}}{D} \quad (9)$$

when  $k_{O_2}$  and  $k_{CO_2}$  represent the diffusion constants of oxygen and carbon dioxide respectively, and  $\Delta C_{O_2}$  and  $\Delta C_{CO_2}$  the difference in concentration of  $O_2$  and  $CO_2$ , respectively, across the placental barrier. Since  $A$ , area and  $D$ , distance—the effective dimensions of that barrier—are common to both sides of the equation, it may be reduced to:

$$k_{O_2} \Delta C_{O_2} = k_{CO_2} \Delta C_{CO_2} \quad (10)$$

or

$$\frac{k_{O_2}}{k_{CO_2}} = \frac{\Delta C_{CO_2}}{\Delta C_{O_2}} \quad (11)$$

The diffusion coefficients for carbon dioxide and oxygen in water are, respectively, —1.378 and —1.607 millimols per square centimeter per day.<sup>11</sup> Substituting these values in equation 8

$$\frac{1.607}{1.378} = \frac{\Delta C_{CO_2}}{\Delta C_{O_2}} \quad (12)$$

and

$$\frac{1.607 \Delta C_{CO_2}}{1.378} = \Delta C_{CO_2} \quad (13)$$

According to our earlier studies<sup>8</sup> the difference in oxygen pressure in the maternal and fetal plasmas of the gravid sheep is about 40 mm. Hg through the last one hundred days of gestation. This pressure difference is equivalent

to a difference in concentration of the dissolved gas of 0.053 millimols per liter of plasma.

(Solubility constant of  $O_2 \times$  oxygen pressure in mm. Hg or  $0.00132 \times 40$ ).

$$\text{Substituting: } \frac{1.607 \times 0.053}{1.375} = 0.061 \quad (14)$$

This, 0.061 millimols, is a difference in concentration that is about one-fourth of our observed value. The inference is that with respect to the exchange of the respiratory gases the tissue barrier of the placenta cannot be regarded as a simple water layer. At the moment no explanation of the discrepancy between the observed and the calculated values is at hand, but the possibility that the solubilities of oxygen and carbon dioxide are relatively different in the placental barrier than they are in water should not be overlooked.

The second question to which we directed our attention was: are the observed differences in the concentrations of the bicarbonate and hydrogen ions of the fetal and maternal plasmas in accord with the view that the placental barrier acts as a semipermeable membrane impermeable to the proteins and permeable to all the ions quantitatively more important such as  $Na^+$ ,  $K^+$ ,  $Cl^-$ , and  $HCO_3^-$ ? If such is the case, the concentrations of these ions on the two sides of the membrane should be in accordance with a Donnan ratio.

The Donnan ratio,  $r$ , across the placenta in the sheep may be estimated from the following data: (i) the colloidal osmotic pressures of the fetal and maternal plasmas and (ii) the mean molecular weights of the fetal and maternal plasma proteins. In our calculations the following assumptions are made: (i) that the maternal and fetal plasmas are comparable to solutions of proteins on the alkaline side of the isoelectric point and a diffusible monovalent electrolyte of a concentration of 150 millimols per kilogram of water, and (ii) that the difference between the colloidal osmotic pressure as measured and the pressure exerted by the proteins per se, as calculated from the mean molecular weight and the concentration of the plasma proteins, is due to the unequal distribution of the diffusible ions.

The difference in concentration ( $C_m$ ) due to the unequal distribution of the diffusible ions between the maternal plasma and an outside solution of the same composition but free of protein is:

$$150 \left( r_m + \frac{1}{r_m} - 2 \right) = C_m \quad (15)$$

where  $r_m$  stands for the Donnan ratio between the two solutions. The

corresponding concentration difference ( $C_f$ ) when fetal plasma is used will be:

$$150 \left( r_f + \frac{1}{r_f} - 2 \right) = C_f \quad (16)$$

where  $r_f$  represents, as in the case of the maternal plasma, the Donnan ratio between the solutions. When the two plasmas are separated from each other by a semipermeable membrane the difference in ionic concentration on the two sides due to the Donnan effect ( $C$ ) will be:

$$C_m - C_f = C \quad (17)$$

and the Donnan ratio between the two solutions ( $r$ ) will be:

$$\frac{r_m}{r_f} = r \quad (18)$$

The colloidal osmotic pressures of the fetal and maternal plasmas of goats and sheep have already been reported.<sup>8</sup> The mean molecular weight of the proteins of the plasma of the maternal sheep has been estimated from the data of McCarthy.<sup>7</sup> The protein concentration of the maternal sheep plasma has been assumed to be 90 grams per kilogram of water; the mean molecular weight is 100,000 and the osmotic pressure due to the proteins alone corresponds to a concentration of 0.9 milliosmols. The colloidal osmotic pressure of the maternal plasma corresponds to a concentration of 1.45 milliosmols. Accordingly:

$$C_m = 1.45 - 0.9 = 0.55 \quad (19)$$

and

$$r_m + \frac{1}{r_m} = 2.00367 \quad (20)$$

$$r_m = 1.0625 \quad (21)$$

The mean molecular weight of the fetal plasma proteins is 65,000. Inasmuch as the concentration of the proteins of the fetal plasma increases with age, the value of  $C_f$  and therefore of  $C$  changes with advancing development. The approximate values for the protein concentration for the different ages are given in Table 2 together with corresponding  $C_f$  and  $r_f$  values.

At pH 7.4 the proteins of both the fetal and maternal plasmas are both on the alkaline side of their isoelectric points. The protein concentration is higher on the maternal side and  $C_m$  is greater than  $C_f$ . Therefore the positive ions should be at higher concentration on the maternal side and the negative ions at the higher concentration on the fetal.

TABLE 2

| Fetal age | Protein conc. gr/Kg water | H <sub>2</sub> O per liter of plasma ml | Prot. con. g % | COP 38° C. mm H <sub>2</sub> O | COP milliosmols | Osmotic pressure of proteins alone | C <sub>i</sub> ' | r <sub>i</sub> ' | C    | r     |
|-----------|---------------------------|---|----------------|--------------------------------|-----------------|------------------------------------|------------------|------------------|------|-------|
| 60        | 20                        | 970                                     | 1.94           | 90.5                           | 0.343           | 0.307                              | 0.0364           | 1.016            | 0.51 | 1.046 |
| 80        | 24                        | 965                                     | 2.32           | 112.0                          | 0.425           | 0.368                              | 0.0565           | 0.019            | 0.48 | 1.042 |
| 100       | 32                        | 960                                     | 3.07           | 157.0                          | 0.595           | 0.491                              | 0.1044           | 1.027            | 0.45 | 1.035 |
| 120       | 43                        | 945                                     | 4.06           | 227.0                          | 0.860           | 0.659                              | 0.2010           | 1.037            | 0.35 | 1.025 |
| 140       | 49                        | 940                                     | 4.60           | 270.0                          | 1.023           | 0.751                              | 0.2715           | 1.043            | 0.28 | 1.019 |

Assuming, for purposes of illustration, that between 120 and 140 days' gestation age when the Donnan ratio  $r$  (see Table 2) is 1.025 — 1.019, the bicarbonate content of the maternal plasma to be 26.0 millimols per kilogram of water and its pH at 7.4, according to the above reasoning the bicarbonate concentration in the fetal plasma should be  $1.025 \times 26$  or 26.6, i.e., 0.6 millimols higher than that of the maternal and the pH of the fetal plasma 0.01 units above the maternal.

In this connection, attention may be directed to the observation that the alkali reserve of the maternal and fetal plasmas from animals 111-140 days' gestation age was found to differ by less than one millimol in four out of five cases.<sup>2</sup> This observation is in agreement with the considerations set forth above, i.e., that if the maternal and fetal plasmas were exposed to the same carbon dioxide pressure, their bicarbonate and hydrogen ion concentrations should be approximately equal.

In vivo, due to the continuous production of carbon dioxide by the fetus the concentrations of the physically dissolved carbon dioxide on the two sides of the placental barrier are not allowed to reach equilibrium; the concentration as our data indicate is always about 0.24 millimols per liter higher on the fetal side. Since

$$\text{CO}_2 = \frac{1}{K_1} \cdot (\text{HCO}_3) \cdot (\text{H}) \quad (22)$$

when  $(\text{HCO}_3)$  and  $(\text{H})$  represent the bicarbonate and hydrogen ion concentrations respectively and  $K_1$ , the first apparent dissociation constant of carbonic acid, if the concentration of the physically dissolved  $\text{CO}_2$  is higher on the fetal side,  $(\text{HCO}_3)$  and/or  $(\text{H})$  must be higher on that side. Unless the properties of the system are more complex than we have assumed them to be, it is difficult to visualize a mechanism whereby the side having the higher concentration of physically dissolved  $\text{CO}_2$  could have a lower bicarbonate and/or hydrogen ion concentration.

Assuming, therefore, that in the steady state the bicarbonate and hydrogen ion concentrations on the fetal side must be equal or higher, but not less than those on the maternal, one can calculate that for a difference in the concentration of the physically dissolved  $\text{CO}_2$  of 0.24 millimols on the two sides of the placental barrier, the bicarbonate concentration should be from 0 to 5 millimols higher, per kilogram of water, and the pH from 0 to 0.08 units lower on the fetal side. As can be judged from a study of the data in Table 1, the results of our experiments are in good agreement with the above considerations.

In some cases the pH of the fetal blood was found to be more alkaline than that of the maternal in the corresponding vessel—comparing uterine artery with umbilical vein and uterine vein with umbilical artery—and the bicarbonate concentration has been found to be higher than expected or slightly below (sheep 144 days, Table 1) the maternal level. The results in these cases may be brought into accord with the reasoning outlined above, i.e., that the placental barrier can be considered to act as a simple semipermeable membrane with regard to the physically dissolved  $\text{CO}_2$ , bicarbonate and hydrogen ions—by assuming that the fetal and maternal plasmas were not in a steady state when sampled—that they were being altered or had been altered by some stress acting on the mother.

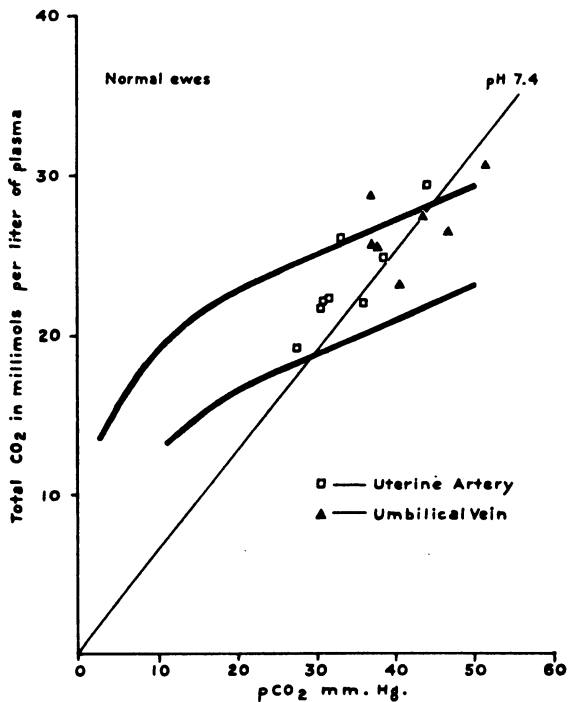


FIG. 1. Graph illustrating the relationship between the total  $\text{CO}_2$  contents of the plasmas of normal ewes and that of their fetuses as obtained in the experiments described above. The heavy dark lines represent the limits of variation in the  $\text{CO}_2$  content of true plasmas of normal ewes whose bloods were sampled directly from the jugular vein without anesthetic or manipulation.

We have observed for example that the anesthetic and procedures associated with the delivery of the uterus frequently result in a fall of several millimols in the total  $\text{CO}_2$  of the maternal plasma—a drop that may occur within the half hour after the beginning of the experiment. Quite frequently the first effect on the mother results in hyperventilation.

That the bicarbonate level of the fetus follows the maternal as the bicarbonate concentration of the cerebrospinal fluid follows the concentration in the plasma,<sup>4</sup> with a delay imposed through the limitation to free diffusion by a tissue barrier, is suggested by a comparison of the data represented in Figures 1 and 2. In the former, the  $\text{CO}_2$  content and pH of the maternal

arterial blood are of such magnitudes as to indicate the alkali reserve of each individual was within or above the normal range found to be characteristic for pregnant ewes.<sup>9</sup> In these individuals the total CO<sub>2</sub> of the fetal plasma tends to be slightly higher and toward the upper limit of the normal range for the mothers. When the ewes are acidotic, i.e., the total CO<sub>2</sub> of the maternal plasma is below the normal level—that of the fetal plasma tends to be lower as well. The delay with which the bicarbonate level of the fetus follows that of the mother is the subject of current investigation.

#### SUMMARY

The concentrations of the dissolved CO<sub>2</sub>, the bicarbonate and hydrogen ion in the plasma drawn from each of the four principal vessels serving the placenta of the sheep and goat—i.e., the uterine artery, uterine vein, umbilical artery, umbilical vein—have been determined at several stages

during the gestation period. The data indicate that the concentration of the dissolved CO<sub>2</sub> on the fetal side of the placental barrier is about 0.24 millimols per liter higher during the last month of the gestation period.

During the same period the bicarbonate concentrations of the fetal plasma ranged from  $-0.17$  to  $+7.9$  mM/l and its pH from  $-0.11$  to  $+0.08$  of a unit with respect to the corresponding values in the related maternal uterine vessels. This variability in the pH and bicarbonate values of the fetal plasma with respect to the maternal is probably due to the circumstance that a sampling of the maternal and fetal bloods in a steady state condition is

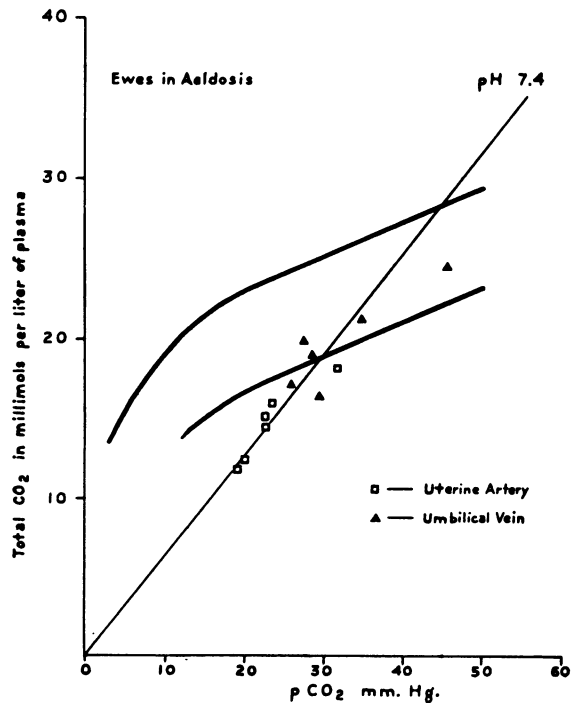


FIG. 2. As in Figure 1 except the ewes were in a state of acidosis as shown by the fact that the total CO<sub>2</sub> content of the maternal plasmas was in each case below the normal range.

rarely achieved. What the bicarbonate and pH values of the fetal plasma with respect to the maternal are in the steady state is discussed.

The concentration gradient of the dissolved CO<sub>2</sub> between the fetal and maternal plasma as reported here has been used as a basis for speculation about the physical properties of the placental barrier.

#### REFERENCES

1. Barcroft, J. and Barron, D. H.: Observations upon the form and relations of the maternal and fetal vessels in the placenta of the sheep. *Anat. Rec.*, 1946, *94*, 569.
2. Barron, D. H.: A comparative study of the alkali reserve of normal, pregnant and fetal sheep. *Yale J. Biol. Med.*, 1953, *216*, 119.
3. Barron, D. H. and Alexander, G.: Supplementary observations on the oxygen pressure gradient between the maternal and fetal bloods of sheep. *Yale J. Biol. Med.*, 1952, *25*, 61.
4. Collip, J. B. and Backus, P. L.: The alkali reserve of the blood plasma, spinal fluid and lymph. *Amer. J. Physiol.*, 1920, *51*, 551.
5. Huggett, A. St. G.: Foetal blood-gas tensions and gas transfusion through the placenta of the goat. *J. Physiol. (Lond.)*, 1927, *62*, 373.
6. Keys, A. B.: The carbon dioxide balance between the maternal and fetal bloods in the goat. *J. Physiol. (Lond.)*, 1934, *80*, 491.
7. McCarthy, E. F.: The osmotic pressure of foetal and maternal sera in the sheep. *J. Physiol. (Lond.)*, 1938, *93*, 81.
8. Meschia, G.: Colloidal osmotic pressures of fetal and maternal plasmas of sheep and goats. *Amer. J. Physiol.*, 1955, *181*, 1.
9. Roos, J. and Romijin, C.: Problems of fetal respiration in the cow—Second part. *Proc. Kon. Ned. Akad. Wet.*, 1940, *43*, 21.
10. Rosenthal, T. B.: The effect of temperature on the pH of blood and plasma in vitro. *J. biol. chem.*, 1948, *178*, 25.
11. Verduin, J.: Diffusion constant and diffusion coefficient. *Science*, 1955, *121*, 215.
12. Van Slyke, D. D., Sendroy, J., Jr., Hastings, A. B., and Neill, J. M.: Studies of gas and electrolyte equilibria in blood. X. The solubility of carbon dioxide at 38° C. in water, salt solution, serum and blood cells. *J. biol. Chem.*, 1928, *78*, 765.