

**Physiology.** — *Problems of foetal respiration in the cow.* By J. ROOS and C. ROMIJN. (From the Laboratory for Veterinary Physiology of the State University, Utrecht.) (Communicated by Prof. G. KREDIET.)

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### Second Part.

#### I. *The oxygen- and the carbon dioxide content of maternal and foetal blood.*

An important point in the problem of foetal respiration is the volume of oxygen and carbon dioxide contained by the foetal blood before passing the placental vessels, and after having been ventilated there.

Such determinations have been carried out by various workers from which COHNSTEIN and ZUNTZ (1884) were the first, and using various experimental animals: the sheep, the goat and the cat. Also for the new born child, born either normally or delivered by Caesarian section, some figures have been given. Most of this work was discussed in an earlier paper (1938).

Attention was also paid to the considerable differences in oxygen saturation of arterial foetal blood, amounting to some hundreds p.c., which had been found by the different authors, whilst especially the low figures for oxygen saturation found in literature had been mentioned. The lowest degree of oxygen saturation found in literature was 17—18 p.c. STEELE and WINDLE (1939) found in the foetal cat a still lower oxygen content, ranging between 0.3 and 4.8 vol. p.c. for arterial blood. Their highest percentage of saturation remained below 45 p.c. In the greater part of their experiments it was below 18 p.c. and even figures below 3 p.c. saturation are given. In a recent paper BARCROFT, KENNEDY and MASON (1940) put attention to the same points. Their criticism is justified for the greater part. The review, given by them, shows that the highest values had been obtained by us in 1938. We had measured in the foetal arterial blood of the cow an oxygen saturation up to 90 p.c., 81 p.c. being the highest value found by others.

Besides this the relation between maximum and minimum, found by the same author in various experiments, by which relation the constancy of observation is expressed to a degree, varied considerably in some papers. Whereas in our work it was as 1.7 : 1, the ratio up to 5 : 1 was found in the work of others. Also the values found for the goat by BARCROFT, FLEXNER and MCCLURKIN (1934), which work had not been mentioned by us in a previous paper, exhibited differences of more than 100 %, the maximum and minimum being 8.50 vol. p.c. and 3.93 vol. p.c. respectively.

Nevertheless our results had not satisfied us fully and new experiments in the cow seemed desirable for many reasons. In the first place because of technical difficulties, experienced in the experimental work. The foetus had been taken out of the uterus, its cord remaining in connection with the placenta. In this way the uterus had been brought under unphysiological conditions. It will also come to contraction and the state of the capillaries of maternal and foetal cotyledones may thus be altered materially. Furthermore it was an open question how the general blood pressure was affected by opening the abdomen and by exposure of the abdominal organs, the uterus especially, to the temperature of the room and to exsiccation. In his fine work on the goat and the sheep BARCROFT has conquered many of the difficulties by emerging the mother in a bath of saline, maintained at a temperature between 37° and 38° C. For the cow, which is a favourable experimenting animal in other respects, this method of working is not practicable. Another technique had to be searched therefore.

#### *Technique.*

The technique, used in the experiments which will be described here, was as follows. After the pregnant cow had been placed on its left side the abdominal wall was opened under local anaesthesia. As we had also done in earlier experiments this form of anaesthesia was preferred to other ones on account of the condition of the nervous system remaining unaffected in this way. The place of incision was chosen in correlation with the position of the foetus, determined by palpation; in most experiments the wound was made about parallel to the midline and about 10 cm higher, that is at the right side of it. It was kept as small as possible, long enough to permit the hand to pass in order to detect the position of the foetus. Then it was lengthened to about 20 cm. In all instances the great curvature of the gravid uterus horn was found to lie near the abdominal wound.

Abdominal wound and uterus were irrigated continually by a 0.9 p.c. salt solution of a temperature between 38° and 39° C. during all the experimenting time. The first sample of blood was withdrawn from a uterus vein, which could be traced through the peritoneum of the uterus. This was done without difficulties. In some experiments another sample was taken from a cotyledone vein which had been exposed at the base of a maternal cotyledone. Then uterus and amnion were opened at the great curvature by an incision between the cotyledones, 6—8 cm long which gave part of the amnion liquor occasion to escape. The further procedure was determined by the situation met. It was our intention to bring part of the umbilical cord at the level of the uterus opening in order to insert a canula. Sometimes however it was not possible to do so without enlarging the wound and bringing one of the foetal hindlegs, some-

times both, outside the uterus. If this was necessary, opening of the allantois could not be avoided. When the latter could be left undamaged the advantage was gained, that a considerable part of the uterus liquor remained inside. As is generally known, the volume of the amnion fluid is reduced gradually after the midst of gestation, whereas the allantois liquor increases and reaches a maximum (up to 10 liter) at the end of pregnancy. At this moment there is still about 4 liter of liquor contained by the amnion.

In some experiments a blood sample was taken from the foetal placenta vessels at the same time. We preferred to take a sample of blood from the umbilical cord because of this blood being mixed and being therefore less susceptible to local alterations in the placental circulation which could have their localisation in some cotyledones or even in a single one.

The insertion of a canula into a vessel of the umbilical cord can not have any undesired effect, since in the cow these vessels, arteries and veins, are paired, and their diameter is such that circulation is not impeded by this procedure in any way. Cooling as well as exsiccation of the cord were avoided by irrigation and a visible contraction of one of the umbilical vessels was never observed.

Further, maternal blood was drawn from the art. carotis and from the jugular vein. As a rule the samples of arterial blood were taken together from mother and foetus; the same was done with respect to the venous blood.

In one experiment (no. 32) in which the calf had been taken out of the uterus and brought under warm salt solution, the uterus remaining under continual irrigation, the results were less satisfactory. In an other one (no. 34) the abdomen was opened at nearly its highest point, viz. in the flank region. Then the uterus, after having been fixed by sutures to the margins of the abdominal wound, was opened. In this way the loss of fluid was restricted to a minimum. The umbilical cord was quickly brought into the wound and samples were taken. The results however were not so good as could have been expected.

An experience, repeatedly made, was that the first factor which was obviously decisive for the results in this work was the tempo of the experiment. The best results were obtained in those experiments which had been carried out with the most push. The time, elapsing between the moment of the opening of the abdomen and the sampling of the foetal blood, was in the good experiments never longer than 10 minutes, sometimes shorter than 5 minutes. Samples, taken 15 min. later intentionally, revealed a lower oxygen saturation. The experience demonstrates an experimental difficulty.

The blood was collected under paraffine oil and thoroughly mixed with either the anti-coagulant powder or the solution, in the manner described in the First Part of this paper (1940). The blood samples were kept in the refrigerator up to the moment of analysis, which was carried out

within an hour. The blood was analysed in VAN SLYKE'S constant volume apparatus, using 2 cc of blood.

### Results.

Eight animals were used for the experiments. In two of them arterial blood pressure was recorded during the experiment. The surprising result was obtained that the pressure was not altered in any way neither by opening the abdomen nor by manipulations in the uterus. An average pressure of 150 mm Hg was maintained.

Four experiments which beforehand we had thought to be the best on behalf of the fluent course of the operation (group A) will be reported here. Besides these the figures, measured in two other cases will be given in table 1 together with those, obtained in earlier experiments.

TABLE 1. Oxygen in the foetal blood of the cow.

Group	Number of animal	Age of foetus in months	O <sub>2</sub> in vol. p.c.		O <sub>2</sub> — Capacity	Saturation p.c.		Ratio umb. art. umb. vein
			umb. vein	umb. art.		umb. vein	umb. art.	
A	38	7	9.07	2.39	10.39	87.3	23	0.27
	36	8	6.76	3.66	10.82	62.5	33.8	0.54
	37	8	4.84	2.25	9.76	50.0	23.1	0.46
	39	8	7.85	4.28	13.10	60.0	32.6	0.54
	Average	—	7.13	3.15	11.02	65.0	28.1	0.43
	33	4	3.43	0.78	11.38	30.1	7	0.23
	34	7	5.19	1.05	9.50	54.6	11	0.20
B	1	7	8.92	2.41	12.15	73.4	19.7	0.27
	2	8	7.48	4.39	11.68	64.0	37.6	0.56
	3	8	6.20	3.46	11.49	54.6	30.4	0.56
	4	8	7.61	2.87	15.57	48.8	18.4	0.38
	5	8½	9.86	5.07	11.35	90.0	46.3	0.51
	Average	—	8.01	3.64	12.45	64.3	29.2	0.45
Sheep 18—21 weeks (Barcroft)			10.5	5.8	16.6	63.3	34.9	0.55

The oxygen saturation, found in experiment 33, was so low that we do not rely on it, though the experimental work had proceeded well. In experiment 34 the uterus had not lost liquor, since it had been attached to the abdominal wall. Nevertheless the results did not satisfy.

Experiment 38 was performed in an animal with hydrallantois, a con-

dition in which an uncommon great volume of liquor is contained by the allantois membrane. The uterus was in a condition of inertia and it contracted only slightly after the foetus had been taken out of it after the experiment. The foetus was a little more slender than it is commonly but it was normally developed.

The oxygen saturation, observed in the experiments 36, 37 and 39, in which the foetus had the age of 8 months, agree rather well for the arterial as well as for the venous blood. On the whole the figures found for saturation are not higher than those observed in earlier work (group B). The degree of oxygen saturation, measured by BARCROFT, KENNEDY and MASON in the sheep at a corresponding stage of pregnancy, does not differ notably from ours, though their figures for oxygen content are somewhat higher. We found an average saturation of 65 p.c. for the arterial and of 28.1 p.c. for the venous umbilical blood, which values are 63.3 and 34.9 respectively in the sheep.

For foetus of the same age the ratio  $\frac{\text{saturation venous blood}}{\text{saturation arterial blood}}$  given in the last column of the table is rather constant.

The figures for the carbon dioxide content are collected in table 2.

TABLE 2. Carbon dioxide content of the foetal blood in the cow.

Number of animal	Age of foetus in months	CO <sub>2</sub> in vol. p.c.		Difference in vol. p.c.
		umb. vein	umb. art.	
38	7	52.78	55.82	3.04
36	8	49.48	51.48	2.00
37	8	41.81	46.44	4.63
39	8	44.72	46.18	1.46
33	4	58.43	60.37	1.94
34	7	52.76	57.07	4.31

Under the conditions of the experiment the oxygen saturation of the arterial blood of the mother varied from 81 p.c. to 93.9 p.c. in the eight animals. The saturation of the blood taken from the jugular vein may differ considerably. It ranged between 23 and 64.7 p.c., 2 observations remaining below 40 p.c. A higher degree of saturation was found in the blood taken from a uterus vein than in that drawn from a cotyledone vein of the same animal. These results are given in table 3.

#### *Discussion.*

How far may the values measured in the foetus be esteemed reliable as approximating physiological conditions?

TABLE 3. Oxygen saturation of maternal blood in the cow.

Number of animal	Oxygen saturation in p.c.			
	a. carotis	v. jugul.	v. uterin.	v. cotyledon
38	93.4	58.8	41.2	—
36	93.9	40.0	57.0	—
37	89.0	41.5	71.1	56.6
39	91.0	51.2	60.5	43.0

This question so far as the oxygen is concerned has also been discussed by BARCROFT, KENNEDY and MASON in their recent paper (1940), in which the authors reported experiments made under controlled conditions in the sheep during a great part of the period of pregnancy, covering from the 63rd to the 145th day. (The period of gestation for the sheep takes 21 weeks.)

Our experiments give only information for the two last months of gestation. The values, found in 3 foetus of 8 months, agree very well; they differ 25 p.c. maximally. The range for the venous blood is somewhat greater but also here the agreement is satisfactory. It even exceeds the degree of constancy found by BARCROFT et al. during the corresponding stage of pregnancy. Neither was there more agreement between their duplicated measurements in younger foetus, in which a higher degree of constancy was stated by the authors. In BARCROFT's experiments, which were made under almost ideal conditions, the blood samples were drawn without taking the foetus out of the uterus and without touching the umbilical vessels which, in the sheep, had been found to be very sensitive for mechanical insult.

A still more valuable standard to judge the value of an experiment is the degree of oxygen saturation found. For duplication of measurements does not exclude the possibility that successive experiments involve the same experimental error. We therefore considered always those experiments to have the greatest chance to be reliable, which gave the highest percentages of oxygen saturation, not only for the arterial blood but also for the venous, and stated already two years ago (1938) that in our experiments the foetal blood, venous as well as arterial, was saturated to a rather high degree. For this reason we gave in the last column of table 1 the ratio  $\frac{\text{oxygen saturation in art. umb.}}{\text{oxygen saturation in v. umb.}}$ . In the 8 months' foetus mentioned under group A this ratio is rather high and fairly constant. Taking also into consideration the experiments of the B-group we have two observations in foetus 7 months old, in which not only the figures for oxygen saturation are rather high and agree in a satisfactory way,

but moreover both ratios given in the last column, agree rather fairly. For both it is 0.27.

Although we think it justified to consider the figures given here approximating physiological values we remain fully aware of the difficulties in this field of work.

It seems that oxygen saturation of the arterial foetal blood decreases in the 8th month of pregnancy. BARCROFT found the same in the sheep for the last three weeks of intra-uterine life. In the venous umbilical blood however we found a lower saturation in the foetus of 7 months than in those one month older.

A question which still has to be considered, is this. Is it reasonable to assume that the umbilical blood, which is oxygenised in the placenta, will be saturated there to the same percentage, when all foetal conditions as age, weight, position, structure of the placental vessels, foetal oxygen dissociation curve are the same, and supposing that the venous foetal blood arrives in the placenta in completely the same state in all instances? Especially for the ox the question has to be answered in the negative, because of the wide dimensions of the field within which the maternal oxygen d.c.s have their position. If the maternal blood is oxygenised in the lungs to a distinct percentage of 93 p.c. for instance, its oxygen pressure, may vary between 56 and 76 mm Hg. It must therefore be concluded that arterialisation of the foetal blood will distinctly experience the consequences of this wide range, and the degree of oxygen saturation of the foetal blood will thereby show variations due to this circumstance. This is another reason for which we deem duplicated measurements though useful of relative value.

## II. *The tensions of the blood gases.*

Since the oxygen dissociation curves had been determined for both maternal and foetal blood (see First Part (1940) ) the partial oxygen pressures on either side of the placenta can be found by interpolating in these curves the percentages of the oxygen saturation measured. The carbon dioxide pressures may be determined in an analogous way, starting from the carbon dioxide dissociation curves. The figures thus obtained are given in table 4.

In Fig. 1 a scheme is given for the relations established for animal 39, at a gestation of 8 months. Two sets of figures are given for the maternal venous blood, for blood taken from a uterus vein and from a cotyledon vein respectively. The figures for the latter have been placed between brackets.

The scheme given here would also fit the experiments 36 and 37. The foetal aerated blood which leaves the placenta through the umbilical veins has a carbon dioxide pressure, still higher than that in the venous maternal blood, whilst the latter is still able to give off oxygen to the foetal aerated blood because of its oxygen pressure being considerably

TABLE 4. Oxygen and carbon dioxide pressure in maternal and foetal blood.

Number of animal	Age of foetus in months	Blood taken from	Oxygen		Carbon dioxide	
			saturation in p.c.	pressure in mm Hg.	volume p.c.	pressure in mm Hg.
38	7	a. carot.	93.4	83	44.67	26
		v. uterin.	41.2	29	47.94	37
		v. umbil.	87.3	34	52.78	51
		a. umbil.	23.0	14	55.82	60
36	8	a. carot.	93.9	67½	46.57	38
		v. uterin.	57.0	36½	47.11	40
		v. umbil.	62.5	23	49.48	41½
		a. umbil.	33.8	14	51.48	44
37	8	a. carot.	89.0	58½	51.92	32
		v. uterin.	71.1	39	55.42	42
		v. umbil.	50.0	25½	41.81	56
		a. umbil.	23.1	15½	46.44	68
39	8	a. carot.	91.0	70	42.39	38
		v. uterin.	60.5	38	47.56	55
		v. cotyl.	43.0	31	49.60	62
		v. umbil.	60.0	24	44.72	70
		a. umbil.	32.6	18½	46.18	73½

higher. So the gas exchange may proceed all during the time that foetal blood passes along the maternal through the placental capillaries. As is well known both capillary systems are separated in the cow by two layers of epithelial cells, from which the maternal is perforated only at the top of the crypt; here the connective tissue comes in contact with the tropho-

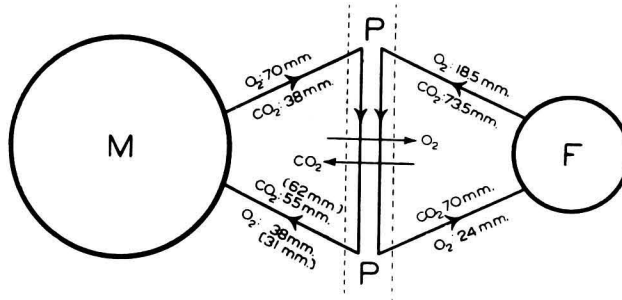


Fig. 1. Gas tensions in the maternal (*M*) and the foetal blood (*F*). Age of the foetus 8 months. *P* = placenta.



blast. So there is not a true placenta but a pseudo-placenta, called placenta syndesmochoriale. A thin layer of uterine milk is found between both epithelial layers.

If the direction in which the blood passes through the foetal capillary vessels should be contrary to that drawn in Fig. 1 the conditions for a continual gas exchange would not be less favourable. Both directions would fit in the scheme and the simplest relation was chosen therefore.

In experiment 38, however, the situation is a different one. Here the conditions, favourable for a good change of gases between maternal and foetal blood are available only then, if the arrangement of the capillary circulation is otherwise.

If we assume that maternal and foetal circulation in the placentae are thus arranged, that foetal ventilation goes on as long as maternal and foetal blood pass each other, we are brought to the scheme of Fig. 2. An other relation is not compatible with the oxygen pressures determined.

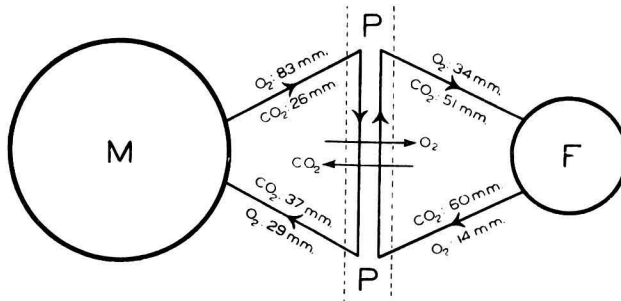


Fig. 2. Gas tensions in the maternal (*M*) and the foetal blood (*F*) in experiment 38. Age of the foetus 7 months. *P* = placenta.

This conclusion is of such importance that it forces us another time to consider critically the figures, from which it is derived. Obviously the direction of the arrows has become imperative by the relative high oxygen pressure in the umbilical veins, which is materially higher than in all other experiments. The figures, found for the carbon dioxide, do not have any preference as to the direction of the arrows.

What may be the reason for this high oxygen pressure? The arterial foetal blood was saturated to a high degree, viz. 87.3 p.c. Probably the state of hydrallantois of the mother was favourable, since there had been little or no tendency to contraction here.

There is still another point of importance for the degree of foetal oxygen saturation: the high oxygen pressure in the arterial maternal blood. It was exceptionally high (84 mm) and surpassed the figures found in all other experiments, which showed an average of 60 mm. This fact finds its origin in two circumstances. Firstly maternal arterial blood was saturated to a rather high degree, 93.4 p.c. In the ox, and the same is probably the case in other animals who do not have a well trained

pulmonary ventilation, a lower degree of saturation is commonly found. Experience showed us that it fluctuates between 80 p.c. and 94 p.c., provided the animal is in lying position, as in the experiments described. When the animal is on its feet other results are obtained. Secondly in consequence of the shape of the oxygen dissociation curve, which has been given in Fig. 10 (o) of the First Part, a high pressure is exerted by the oxygen in the region of high saturation. If for instance the same degree of saturation had been found in experiment 36 (Fig. 11) ( $\Delta$ ) First Part, the oxygen pressure of maternal blood would have been no more than 65 mm, whereas in the experiment in question a pressure of 84 mm is exerted. So it is conceivable that by the high maternal oxygen pressure foetal oxygenation is performed to a high degree.

On the ground of this experiment one is inclined to correct the direction of the arrows, given in Fig. 1 and to presume that in the cow the arrangement of maternal and foetal capillaries in the placenta is thus, that blood circulation occurs as shown by Fig. 2. It remains reserved to further work, to settle this problem. Here we have only to refer the reader to the anatomical work by MOSSMAN (1926) who, by pure anatomical examination, came to the conclusion that in the rabbit placental capillaries are arranged in the manner indicated by the scheme of Fig. 2, so that in the foetal capillaries of the placenta the blood passes in the opposite direction as in the adjacent maternal. According to MOSSMAN, TAFANI would have found before him the same relation in the placenta of the cat. To our regret TAFANI's paper was not obtainable for us.

*Summary of the First Part (1940) and Second Part of this paper.*

Various points of foetal respiration have been examined in the cow. The results are as follows.

(1) During the period of pregnancy the maternal oxygen dissociation curve becomes steeper, and starting from the 4th month its inflection increases. The curve does not leave the field determined for normal non-pregnant cows; its lower most inflected part only may fall outside of it.

These changes are not produced by changes in reserve alkali.

(2) In these respects the curves differ from those determined for the goat and for man.

(3) As a rule the reserve alkali of the pregnant animal does not fall below the level of normal non-pregnant animals.

(4) The foetal oxygen dissociation curve differs from the maternal by both position and shape. It is found to the left of the maternal and to the left of the area, established for normal non-pregnant cows.

(5) The gap between maternal and foetal curve, though brought about without a displacement of the maternal curve, is a considerable one so that a notable difference in oxygen avidity between maternal and foetal blood is expressed by it.

(6) Foetal and maternal carbon dioxide curves have been determined and discussed. The dislocation of the foetal oxygen dissociation curve is counteracted by a fall of alkali reserve in the foetal blood during the last months of foetal life.

(7) The foetus has a haemoglobin with other properties than the maternal.

(8) A fairly constant oxygen saturation of the blood in the umbilical vessels was found in foetus, 7 and 8 months old. It varied between 50 and 87 p.c. for the arterial blood and between 23 and 32 p.c. for the venous. The content of carbon dioxide was also determined. At the same time corresponding measurements have been carried out for the maternal arterial and venous blood in the placenta. The reserve alkali in both mother and foetus has been discussed.

(9) The tension of the gases in both maternal and foetal blood was determined by interpolating the figures for oxygen saturation and carbon dioxide content in the respective dissociation curves. So it was made probable that the arrangement of the capillaries in maternal and foetal placenta is thus that the blood which passes on either side in order to change gases and other materials goes in directions, opposite to each other.

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