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OXYGEN IN THE BLOOD OF THE UMBILICAL VESSELS OF THE SHEEP

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A NUMBER of authors have published particulars concerning the oxygen content and percentage saturation of the blood in various vessels in the mammalian foetus. These results have been characterized by the very great divergence in the values obtained. This divergence applies even to the observations of any one observer and to any one vessel.

The records have fallen into three main categories, concerned respectively with (1) the comparison of the maternal and foetal bloods, (2) the metabolism of the foetus and (3) the comparison of the foetal carotid blood with that in the umbilical vessels.

Though the errors likely to appear in the records vary a little according to the object for which the experiments are performed, we may for the present purpose consider them together and indeed it is only necessary to take a single vessel, the umbilical vein, to exemplify the divergence which applies to all (Table I).

TABLE I. Oxygen in blood in umbilical vein

Author	Animal	Content vol. %	Percentage saturation. The figures in brackets are approximate, calculated from an assumed oxygen capacity: goat = 16, dog = 20
Cohnstein & Zuntz [1884]	Sheep	6·3	42
Barcroft, Flexner & McClurkin [1934]	Goat	8·5–3·9	81–(33)
Roos & Romijn [1938]	Cow	9·8–6·3	90–53
Haselhorst & Stromberger [1930, 1931]	Man (normal birth)	14·9–5·3	45–33
	Man (Caesarean)	5·7–2·5	
Eastman [1930]	Man (in utero)	13·3 average	63
	Man (at birth)	13·2–8·2	50
		10·5 average	
Huggett [1927]	Goat	12·5	(80–31)
Kellogg [1930]	Dog	11·8–2·1	(60–11)
Steele & Windle [1939]	Cat	8·6–0·3	75–(3)

The statements from this laboratory on the subject of the oxygen in the umbilical vein have been very guarded. A curve was given by Barcroft, Elliott, Flexner, Hall, Herkel, McCarthy, McClurkin & Talaat [1935] which showed a gradual rise from 60 to 87 % saturation in the weeks between the 70th and 115th days, with an abrupt fall in the last week. Our own comments on this curve at once show our caution and the reason for the present research. "None of our observations has given us more solicitude than those of direct records of the percentage saturation of blood taken from the umbilical vein. The results vary greatly but Fig. 1 gives the upper limit. Often the points are much lower. At present we have not sufficient information to discuss fruitfully whether these lower points are due to experimental procedure, e.g. the constriction of the uterine vessels when the uterus is opened." But while it is suggested above that the lower points are due to experimental error, it does not follow that the higher ones are free from it. Thus as may easily be demonstrated, if the maternal circulation in the placenta is good and the foetal circulation is restricted, the blood leaving the placenta along the umbilical vein will become bright red even if it were not so before. In that case the blood in the umbilical artery will be correspondingly dark and the oxygen difference great.

TECHNIQUE

Our experiments have been done on sheep. The principal source of error seemed likely to be the exposure of the cotyledons to something different from their normal environment. It was therefore decided to draw the samples if possible without taking the foetus out of the uterus.

This proved to be easy in the case of the umbilical artery and the umbilical vein, especially when dealing with singlets. In such cases the blood from both horns of the uterus contributes to the general flow, and through an incision in the wall of the horn not occupied by the foetus blood may be taken from branches of the main artery and vein. There is as a rule no difficulty in obtaining blood from the carotid, or from the jugular vein without taking the foetus out of the uterus. The secret lies in fixing the head, otherwise the foetus is very elusive. On inspection of the uterus, before any cut is made in its wall, it is often possible to locate a projection caused by an ear. A stitch is put through the wall swiftly, and the pinna is sewn to the uterus. The wall is then cut open over what is judged to be the position of the jugular vein, the skin of the foetus is opened in the same position and the lips of the two wounds are clipped together so that in effect the uterine wall becomes part of the foetal integument. A cut is made along the posterior edge of the jugular, then by suitable pressure from below (after a little practice) there is no difficulty in forcing up the carotid into the wound so made.

When as in our later experiments the mother is under a spinal or local anaesthetic, the above operation on the foetus may be done under a local anaesthetic. Up to the hundredth day or thereabouts the above precautions suffice, but after that date another factor becomes very important, namely the position of the uterus. The foetus has obtained a weight of upwards of a kilo. and it is very easy to interfere with the maternal circulation by undue pressure on the larger vessels. Therefore the paramount object must be to keep the uterus in its normal position. For instance, if to obtain carotid blood it becomes a choice between moving the uterus and withdrawing the head of the foetus, the latter would be the procedure.

The observation of these precautions offers no great difficulty until after about 130 days; then the whole operation becomes so fraught with pitfalls that, even after doing it many times, we never approach it without the feeling that we are at best facing an almost impossible task.

The reason why the operation becomes so difficult is of course that the operator is in competition with the inherent physiology of the animal. It is part of the very essence of intra-uterine life that at its termination the uterus and the foetus should react to the most trivial interference in a way similar to birth, i.e. primarily by occlusion of the uterine vessels and the cord. The umbilical cord itself becomes much more responsive to touch, to strain, or even to exposure. At an earlier stage, the dropping upon it of a few drops of 1 % formalin will usually suffice to preserve the calibre of the vessels, not so as birth approaches. It is necessary at this stage that the cord should never be pulled, that it should never be out of an isotonic medium and, if possible, that it should not be touched.

What is true of the cord is true of the maternal vessels though in a less degree. The procedure which we have found most successful for this final stage has been as follows: The sheep under a spinal, or perhaps better under a local anaesthetic, is placed in the bath. A V-shaped incision is made from the pubic symphysis; in one direction it goes up the middle line, in the other parallel to and along the abdominal side of Poupart's ligament. The length of these incisions is a matter of judgement, the object being to expose the uterus without exposure of other abdominal viscera; the edge of the omentum is often a good guide. The sheep is now turned over on to its side in the bath. To facilitate this the animal is lying on slings; two for its support pass underneath it, while the third, which is for the purpose of keeping its back hard up against the wall of the bath (so as to give as much room as possible between the abdominal wall and the side of the bath adjacent to it), is passed round the sheep but with both ends brought over the side of the bath against which the back is pressing. A small stand or bed for the foetus to lie upon is suspended from a support which can at once be put in position. The position is along the abdominal wall, at the level of the middle line. The height of the sheep is regulated with the slings so that it is sufficiently, but not more than sufficiently, immersed in the saline (40° C.). The uterus is then opened. At this stage, especially in animals of more than 140 days foetal age, there is little bleeding. The foetus is withdrawn and placed, without subjecting the cord to any strain, on its stand. Notwithstanding these precautions there is always the fear of the aeration of the blood becoming less efficient as the time passes and it is always well to take the samples as soon as possible. In the case of the umbilical vessels this should be within about a minute of opening the uterine wall. In the case of the carotid, the dissection needs about 5 min., but of that more will be said in a future paper.

We use any good syringe, but the needle demands care, the larger the bore of the needle the quicker the samples can be obtained, the smaller the bore the less the trauma. Our principle has been to take the smallest needle consistent with the withdrawal of the blood in about 3 sec. In very young foetuses the blood should be withdrawn more slowly lest the general pressure relations, especially the circulation in the heart, be upset; a useful syringe for the purpose is one of which the plunger screws out. The point should be very sharp. Among the methods of damaging the cord, one of the most effective is the endeavour to pierce its wall with a needle, the point of which is not of a high degree of sharpness. Of course, the smaller the needle relatively to the lumen the less the interference with the blood flow. The blood samples were analysed in the Van Slyke manometric apparatus, making use of Mason's [1939] technique for the delivery of small samples. Usually 0·2 c.c. were taken for analysis. The possible errors introduced by using such samples are small and completely negligible as compared with the physiological errors caused by manipulation and the loss of blood in the case of animals younger than 80 days.

The sample when taken is at once transferred to a small container in which is heavy liquid paraffin and under that an anti-coagulant, either in the form of liquid or powder. It is important that this anti-coagulant should kill the blood, i.e. that it should inhibit the glycolytic power of the blood [Evans, 1922] and so destroy the power of the blood to reduce itself. When a solution was used, it had the following composition:

$(COOK)_2\ H_2O$	3 g.
NaF	1 g.
NaOH *M*/100	1 ml.
Water to	100 ml.

When the powder was used, it consisted of a mixture of sodium fluoride and potassium oxalate in the proportion of 1 : 3 by weight. Of the powder 5–15 mg. were taken per c.c. of blood, more being required for younger foetuses. As the blood can only be stirred slowly lest it should emulsify with the paraffin, traces of heparin powder were also added.

The containers were kept in ice till the analysis could be made.

The only series of observations in which an attempt has been made to follow the oxygen content and percentage saturation systematically throughout pregnancy has been carried out in this laboratory on sheep and some of the results have been published [Barcroft, 1938]. These results indicate a general tendency, as pregnancy proceeds, for the percentage saturation to fall in the umbilical artery, the umbilical vein and the carotid artery. The figures obtained were however very irregular; for instance, two foetuses on which the determinations were carried out at approximately the same period of pregnancy gave the following results:

		Oxygen saturation (%)	
Sheep	Foetal age days	Umbilical vein	Umbilical artery
19	137	42	10
27	138	75	43

If in the placenta the maternal flow was unduly slow and the foetal flow was not, one might expect a result such as that shown by sheep 19 in which the foetal blood was poorly oxygenated. If on the other hand the foetal flow was unduly slow and the maternal flow normal, the foetal blood would probably arrive at the placenta very dark but would leave it very bright, and such cases have been observed. It is not to be assumed that because the blood in the umbilical vein is bright red that the conditions are satisfactory. Such independent alterations in the relative flows of the maternal and foetal bloods through the placenta may be physiological and it is not unreasonable that so great a degree of irregularity should exist; on the other hand before accepting it, it seemed desirable to carry out observations under much stricter conditions.

The object of the present paper is to supply records of the saturation and content of the blood in the umbilical and other vessels, which inspire more confidence than those hitherto published, more confidence because obtained under controlled conditions.

THE OXYGEN IN THE UMBILICAL VESSELS

The question we set ourselves was the following: Of a number of determinations taken at different stages of pregnancy and widely different in result which, if any, could we duplicate by the improved techniques which we have described?

In general, the answer was that the greater the precautions taken the more likely we were to duplicate observations in which both the oxygen in the umbilical vein and that in the umbilical artery were high. This at least is so up to about 130 days. (The period of gestation of the sheep is 147 days.)

Table II gives figures for oxygen content percentage saturation, and oxygen capacity of the blood of sheep foetuses up to 145 days.

TABLE II. Oxygen in blood in umbilical vessels

Sheep no.	Foetal age days	Oxygen content c.c./100 c.c. blood		% saturation		Oxygen capacity c.c./100 c.c. blood
		Umbilical vein	Umbilical artery	Umbilical vein	Umbilical artery	
500 S	63	8·0	3·6	85	38	9·4
299 T	66	10·8	2·3	83	18	13·0
340 S	78	10·1	3·4	93	31	10·8
519 S	80	11·4	4·7	98	42	11·6
255 S	83	10·3	6·6	75	48	13·8
286 S	88	13·5	5·8	93	40	14·5
312 S	89	11·7	4·1	87	31	13·4
331 S	96	13·8	9·4	92	62	15·1
459 S	112	12·9	10·0	87	67	14·9
262 S	113	11·4	8·0	83	58	13·7
306 S	113	12·9	8·9	88, 87	60	14·8
345 S	126	8·4	5·9	77	54	10·9
285 S	127	12·5	9·5	77	58	16·3
470 T	139	10·6	4·3	50	20	21·2
444 T	143	12·8	6·2	70	34	18·4
515 S	143	8·6	4·8	48	27	18·0
498 S	144	12·35	4·8	65	25	19·0
572 T	145	8·5	5·3	65	40	12·21

S = singlet; T = twin.

The table seems to show:

(1) That up till near the end of pregnancy (somewhere between the 127th and the 139th day) it has been possible to obtain duplicate readings

from different sheep for the percentage saturation of oxygen in the blood in the umbilical vein with considerable success.

(2) That in this period the percentage saturation is high, using the word high to mean of the same order as has been found in the arterial blood of men who have been conscious, though not necessarily alert. Somewhere in the region of 70 % may be taken as a useful line.

(3) That on the whole the percentage saturation of the blood in the umbilical vein is highest between the 75th and 100th days, after which it gradually falls.

(4) That during the last week of pregnancy the percentage saturation in our experiments drops considerably and the figures become irregular. Some reasons will be given later for the supposition that these irregularities may not be entirely due to our inability to compete with the admittedly difficult conditions of the experiments.

(5) That the percentage saturation in the umbilical artery underwent greater changes than in the umbilical vein. Starting at about 65 days at a quite low figure, it rose at about 95–115 days to values in the region of 60 % saturation, then again fell.

(6) That the oxygen difference expressed in terms of percentage saturation was great in the younger foetuses being of the general order of 50–60 % saturation. It then decreased and from the 96th till the 127th day it was only about 20 % saturation. In the final week it was irregular but it never approached the 60–80 day values.

(7) That apart from one or two anaemic sheep (572 and 345) the oxygen capacity per c.c. of blood followed the course described by Elliott, Hall & Huggett [1934] for the goat, namely it rose in the early stages, remained fairly level from the 80th day until close upon term, when it again rose abruptly.

(8) That the oxygen content of each cubic centimetre of blood in the umbilical vein is more irregular than the percentage saturation; the oxygen content is maintained to the end of pregnancy, the rise in oxygen capacity and the fall in percentage saturation more or less balancing one another.

(9) That the oxygen content of each cubic centimetre of blood in the umbilical artery starts at a low figure in the 60 day stage, rises to a high one at about 100 days and then again sinks.

(10) That the absolute oxygen difference is large in the earliest periods, frequently 7 or 8 c.c./100 c.c. of blood, and drops to 3–4 c.c. between 96 and 127 days. In the final period it is variable.

Discussion

The first question which arises for discussion is: How do the observations of previous workers on the oxygen in the blood of the umbilical vessels appear in the light of the work described above?

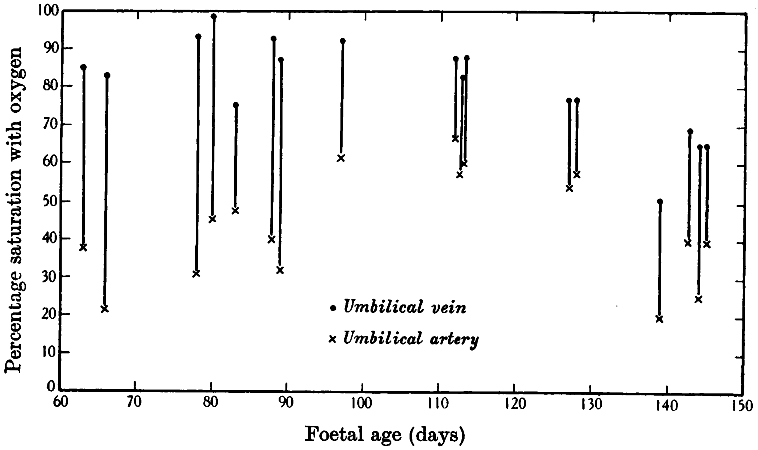

Fig. 1. Percentage saturation with oxygen of blood in umbilical vessels during foetal life.

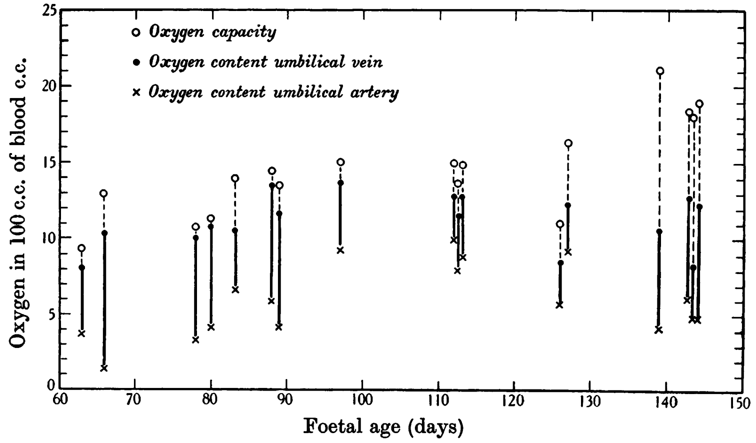

Fig. 2. The oxygen capacity and oxygen content of blood in the umbilical vessels during foetal life.

The answer is perhaps most easily obtained by inspection of Figs. 1 and 2. Comparing Table I with those figures, except perhaps in the case of Kellogg's [1930] determinations, it would be possible to find at some stage of pregnancy a determination in the figures which matched any one in the table. Therefore unless the foetal ages are specified the determinations mean little. On the other hand most of the values given in the table aimed at being near term and as such are probably not far wrong, for at that time the results seem to be inherently variable, the variation being associated, as Steele & Windle [1939] have pointed out, with alterations of tension in the uterine walls.

Where previous observations fall short is that none of them does or can give a picture of the "oxygen in the umbilical blood during pregnancy". The oxygen in the blood during pregnancy undergoes a series of ordered changes which can only be placed in a single picture by the plotting of serial determinations made under very carefully controlled conditions.

Inasmuch as the saturation in the blood of the umbilical vein is, till close upon term, over 75 % and up till 113 days is usually in the region of 85–95 %, the new information gives a very different idea from that given by Table I.

The composition of the blood which supplies the head will form the subject of a later paper but there is no controversy about that which supplies the body; it is identical with that found in the umbilical artery. Till the 90th day the saturation of this blood is low and as the oxygen capacity is only about 10 vol. %, the oxygen content is low also. Comparing the blood at this period with that in the umbilical artery at term, the saturations are of the same order but the content at term is greater owing to the greater oxygen capacity.

A striking feature is the sudden rise in the oxygen in the umbilical vein in both saturation and content at about the 90th day. It is difficult not to associate the rise with certain events which take place in the placenta at that time:

(*a*) The cotyledons attain their maximum weight at that time.

(*b*) The vascular bed in the placenta undergoes a remarkable development about that time. The observations of Barcroft & Kennedy [1939] showed that between about the 80th and the 93rd day the vascular bed in the placenta increased threefold, whilst by the 93rd day the bed was already of the same order of size as at term; the maximal size of the bed seemed to be between the 120th and 130th days (Table III).

It is perhaps premature to pursue this phase of the subject further, pending observations as yet unfinished on the structure of the placenta and the vascular conditions.

TABLE III

		Volume of blood		
Number	Foetal age days	Total c.c.	Foetus c.c.	Placental bed c.c.
268 S	78	53	43	10
340 S	78	52	29	23
264 T	82	53	31	22
266 S	93	136	65	71
333 S	105	220	150	70
298 T	108	180	114	66 R
		189	120	69 L
309 S	111	266	165	99
256 S	112	287	193	94
343 S	118	219	126	93
289 T	124	326	198	128
337 S	125	289	206	83
345 S	126	319	141	178
348 T	133	444	349	95
273 S	140	565	498	67

S = singlet; T = twin; R = right; L = left.

SUMMARY

The information in the paper is summarized in the numbered paragraphs on pp. 352–3.

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REFERENCES

Barcroft, J. [1938]. *The Brain and its Environment*, Figs. 6 and 8. Yale University Press.

Barcroft, J., Elliott, R. H. E., Flexner, L. B., Hall, F. G., Herkel, W., McCarthy, E. F., McClurkin, T. & Talaat, M. [1935]. *J. Physiol.* **83**, 192.

Barcroft, J., Flexner, L. B. & McClurkin, T. [1934]. *J. Physiol.* **82**, 498.

Barcroft, J. & Kennedy, J. A. [1939]. *J. Physiol.* **95**, 173.

Cohnstein, N. & Zuntz, N. [1884]. *Pflüg. Arch. ges. Physiol.* **34**, 173.

Eastman, N. J. [1930]. *Bull. Johns Hopk. Hosp.* **47**, 221.

Elliott, A. H., Hall, F. G. & Huggett, A. St G. [1934]. *J. Physiol.* **82**, 60.

Evans, C. Lovatt [1922]. *J. Physiol.* **56**, 146.

Haselhorst, G. & Stromberger, K. [1930]. *Z. Geburtsh. Gynäk.* **98**, 49.

Haselhorst, G. & Stromberger, K. [1931]. *Z. Geburtsh. Gynäk.* **100**, 48.

Huggett, A. St G. [1927]. *J. Physiol.* **67**, 373.

Kellogg, H. B. [1930]. *Amer. J. Physiol.* **91**, 637.

Mason, M. F. [1939]. *J. Physiol.* **94**, 550.

Roos, J. & Romijn, C. [1938]. *J. Physiol.* **92**, 249.

Steele, A. G. & Windle, W. F. [1939]. *J. Physiol.* **94**, 531.