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arterial oxygen content
choroidal blood flow
fetus
neonate
retinal blood flow

Retinal and Choroidal Blood Flow in Unstressed Fetal and Neonatal Lambs

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Summary

The relationship between retinal blood flow (RBF) and choroidal blood flow (ChBF) and the oxygen content of arterial blood was investigated in 12 fetal lambs. The postnatal changes in these flows were studied in twelve newborn lambs. RBF and ChBF were determined by means of radioactive microspheres 3 to 10 days after implantation of the injection and sampling catheters. Fetal blood flows were measured at different levels of oxygenation. The postnatal flows were measured while the lamb breathed room air. The fetal data suggest an inverse relationship between RBF and arterial oxygen content. Concomitant changes in fetal ChBF were unrelated to arterial oxygen content. Because of the inverse relationship between RBF and arterial oxygen content, the product of RBF and arterial oxygen content was independent of the state of fetal oxygenation.

RBF did not change with birth, whereas ChBF increased. There was no change in RBF with postnatal age whereas ChBF decreased significantly with increasing age.

Speculation

A reduced vascular resistance in the immature choroid might be an important predisposing factor in the development of retrolental fibroplasia in preterm babies treated with high environmental oxygen.

There has been considerable interest in the regulation of retinal and choroidal blood flow as a result of the association of oxygen toxicity and retrolental fibroplasia (9). Dollery *et al.* (6) proposed a model by which excessive constriction of immature retinal vessels might be produced by an increase in tissue PO_2. In their study, however, a number of assumptions made about the retinal and choroidal circulations were derived from acute experiments in anesthetized animals in which physiologic reactance (18) might have altered retinal and choroidal physiology. The goal of the present study was to describe the relationships observed under chronic experimental conditions between the fetal retinal and choroidal blood flows and oxygen contents over a wide range and changes in these flows with postnatal age.

MATERIALS AND METHODS

Twelve pregnant ewes of mixed western breed at 130 to 145 days of gestation and 12 newborn lambs in the first 5 months after birth were studied. Surgery was performed under pentobarbital sedation (5 mg/kg) and, in the ewes, also spinal anesthesia (8 mg tetracaine hydrochloride). As described previously for fetal lambs (12), polyvinyl chloride catheters (ID, 0.9 mm, OD, 1.2 mm) were inserted into a transverse scapular artery, a pedal vein, both pedal arteries, and the amniotic sac. In newborn lambs, the catheters were inserted into a transverse scapular artery, the left ventricle, and a femoral artery. The ends of the catheters in both fetal and newborn lambs were exteriorized via a SC tunnel and stored in a pouch on the flank of the ewes and the newborn lambs, respectively.

MEASUREMENTS IN FETUSES

Studies were carried out between the third and 10th postoperative day. In each animal, three sets of flow measurements were performed by means of the microsphere technique (3, 11, 14) at different levels of fetal oxygenation. Microspheres of 15 μm diameter (3M Co., St. Paul, MN) labeled with either ^{51}Cr, ^{141}Ce, or ^{85}Sr were used. Changes in fetal oxygenation were induced by exposing the ewe to varying concentrations of oxygen (10 to 100%). The different gas mixtures were delivered in random order at high flow rates into a large plastic bag that completely enclosed the head of the ewe. To attain steady-state conditions in fetal oxygenation and cardiovascular function at each gas mixture, a minimum of 30 min was allowed for equilibration. Fetal arterial blood pressure (FBP; torr) defined in this study as the difference in mean pressure between the fetal abdominal aorta and the amniotic sac, and fetal heart rate (FHR; beats/min) were monitored throughout the experiment. Blood flow measurements were performed when stable FBP and FHR had been recorded for at least 20 min. At the time of each flow measurement, blood samples were collected from each of the arterial catheters for measurements of pH, PCO_2, PO_2 (at 39.5°C by means of Radiometer BMS3 MK2), O_2 content, (by means of the Lex-O_2-con), and hematocrit (microcapillary technique). Return to pre-experimental values of both FHR and FBP always occurred after completion of the experiments. The ewe was then sacrificed, and the fetus was delivered by cesarian section. Catheter positions were checked at autopsy. All fetal organs were dissected as described previously (14).

The eyes were enucleated, and separate samples of choroid and retina were obtained by way of the dissection procedure described by Alm and Bill (1). The tissue samples were dried for 24 hr at 80°C, weighed, and γ-radiation was determined with a gamma counter (Nuclear Chicago three-channel autogamma). In three fetal lambs dry weight/wet weight ratios were determined for both retinal and choroidal tissue. For both tissues the dry weight/wet weight ratio was ~0.20, in agreement with observations reported in the literature (1).

For the flow calculations, it was assumed that a dry weight/wet weight ratio of ~0.20 applied to retinal and choroidal tissue throughout the perinatal period studied.

Blood flow to both retina and choroid was calculated according to the following equation:

$$TBF = \frac{CPM_{ts} \times ts \cdot DrW}{CPM_r \times 0.20} \times F_r \qquad (1)$$

where TBF = tissue blood flow (ml·min^{-1}·100 g^{-1}), CPM_{ts} = total cpm in the tissue sample, CPM_r = cpm in the arterial reference sample, ts·DrW = dry weight of the tissue sample, and

F_p = the sampling rate of the withdrawal pump (1.28 ml·min^{-1} in each experiment).

Calculations showed that the retinal samples always contained ≥200 microspheres of a particular label whereas arterial reference samples and choroidal samples always contained ≥800 microspheres. The relatively large variability in retinal blood flow was due, in part, to the low number of spheres in the retinal tissue samples (3). The injection of a higher total dose of microspheres would have reduced this variability, at the expense, however, of more interference with fetal physiology (2, 19).

MEASUREMENTS IN NEWBORN LAMBS

The newborn lambs were studied between the second and fifth postoperative days. Their postnatal age at the day of experimentation ranged between 2 and 226 days. In most instances, flow measurements were made with the newborn lamb sitting quietly in a large cardboard box breathing room air. To reduce potentially disturbing factors such as excessive light or noise, the cover of the box was loosely closed, and the ends of the three catheters were brought outside through a hole in the top of the box. Blood flow measurements were performed as described for fetal lambs. At the time of each flow measurement, the arterial blood pressure (torr) was measured as the difference in height between the fluid level in the transverse scapular arterial catheter and the xiphoid process. After the third microsphere injection, the newborn lambs were sacrificed, and tissue was processed as described for the fetal lambs.

DATA ANALYSES

For fetal lambs, the relationship between blood flow and oxygen content in the ascending aorta ($[O_2]_a$; mM) for both retina and choroid was analyzed as follows. In general, three blood flow values were obtained from each animal. A mathematical model was chosen for the description of the flow-oxygen relationships for the retina and choroid which allowed each animal to have its own "animal effect" whereas the empirical equation was identical for each of the twelve animals studied. A major advantage of this approach over an analysis based on pooled data is that the effect of oxygen on organ flow can be analyzed, whereas possible effects of differences between animals, particularly those due to systematic experimental error, are filtered out. The flow-oxygen relation for both the retina and the choroid could be described by the general equation:

$$\underline{y}_{ij} = \mu + \alpha_i + \beta f(\gamma, x_{ij}) + \underline{\varepsilon}_{ij} \qquad (2)$$

where $\underline{y}_{ij}$ = flow measurement in the j^{th} experiment on the i^{th} animal; μ = a constant, identical for all animals; α_i = additive "animal correction factor" due to the i^{th} animal. (Correction factor α was determined in such a way that the sum of the twelve values for α equaled zero); β, γ = regression coefficients, assumed to be the same for all animals; x_{ij} = value of $[O_2]_a$ in the j^{th} experiment on the i^{th} animal; $f(\gamma, x_{ij})$ = transform of x_{ij} and γ (*e.g.*, $f(\gamma, x_{ij}) = e^{\gamma x_{ij}}$, etc.); $\underline{\varepsilon}_{ij}$ = error term with expectation of zero and standard deviation proportional to the expectation of $\underline{y}_{ij}$.

The mathemathical elaboration associated with the analysis described above is given in detail in the appendix to this report and is termed "weighted least-squares method" in the remainder of this publication.

RESULTS

FETAL STUDY

The flow data and concomitant arterial parameters in the twelve fetal lambs used in this study are listed in Table 1. For reasons mentioned elsewhere (13), O_2 content rather than PO_2 was used as index for oxygenation in the flow-oxygen relationships studied. The relationship between RBF and $[O_2]_a$ is presented in Figure 1. By the weighted least-squares method, a number of mathematical

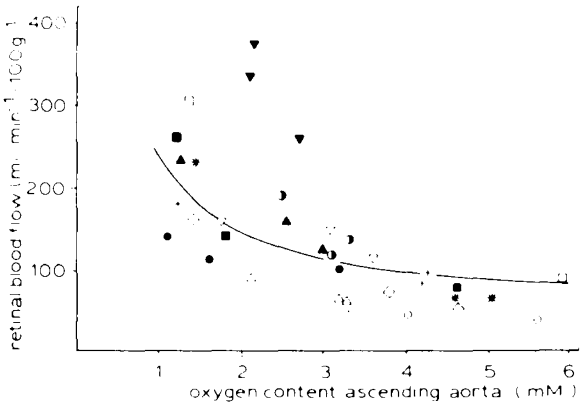

Fig. 1. Relationship between fetal RBF and $[O_2]_a$ in the ascending aorta. The flow data of different animals are represented by 12 different symbols. *Line* is calculated using the reciprocal function for an animal with animal effect $\alpha = 0$ (see text).

functions were evaluated as to their adequacy to describe the RBF-$[O_2]_a$ relation. The results summarized in Table 2 suggest that some nonlinear function describes that relationship best. However, no clear discrimination could be obtained between the three nonlinear functions tested. In other fetal neural structures such as cerebrum, brain stem, and cerebellum, it has been demonstrated that the flow-oxygen relation was best described by a reciprocal function (15). Therefore, it was assumed that a reciprocal function might best describe the flow-oxygen relation for the fetal retina. Consequently the following equation was selected:

$$RBF = \mu + \beta^{-1}/[O_2]_a + \alpha \qquad (3)$$

By the weighted least-squares method, the parameters in equation 3 were calculated:

$$RBF = -55 + 1981/[O_2]_a + \alpha \qquad (4)$$

In this and all subsequent equations, blood flow is in ml·min^{-1}·100 g^{-1}. The additive animal effect α ranged from 0 to +233. According to the weighted least-squares method, approximately 82% of the intra-animal variability in RBF could be explained by changes in $1/[O_2]_a$.

Equation 4 can also be expressed as follows:

$$RBF \cdot [O_2]_a = 198 + [O_2] \cdot (\alpha - 55) \qquad (5)$$

The equation expresses the relationship between the flow of O_2 to the fetal retina (μmoles·min^{-1}·100 g^{-1}) and the $[O_2]_a$. We were not able to demonstrate a significant correlation between the flow of oxygen to the fetal retina and the level of fetal oxygenation (sign test, $P > 0.1$).

The relationship between choroidal blood flow (ChBF) in ml·min^{-1}·100 g^{-1}) and $[O_2]_a$ is presented in Figure 2. Analysis of this relationship by the weighted least-squares method indicated that fetal choroidal blood flow (ChBF) and $[O_2]_a$ were not correlated. ChBF ranged from 900 to 3700 ml·min^{-1}·100 g^{-1}, with a mean for all fetal data points combined of 1620 ml·min^{-1}·100 g^{-1} (±127 S.E.).

NEWBORN STUDY

The flow data and concomitant arterial parameters in the 12 newborn lambs studied are listed in Table 3. Inasmuch as the level of oxygenation was not altered in newborn lambs, no mathematic function could be constructed for the RBF-$[O_2]_a$ relationship. It is noteworthy, however, that the neonatal data seem to extend the trend shown by the fetal data points in the higher $[O_2]_a$ range (Fig. 3). ChBF was slightly higher in newborn lambs than in fetuses. The values for ChBF ranged from 1550 to 3966 ml·min^{-1}·100 g^{-1} with a mean of 2700 ml·min^{-1}·100^{-1} (±160 S.E.). Comparison of the mean ChBF before and after birth indicated that this value

Table 1. *Retinal and choroidal blood flows with the concomitant values for FBP, as well as PO_2, PCO_2, pH, and $[O_2]_a$ in the ascending aorta of the 12 fetal lambs*

Identifying symbol	Experiment no.	RBF (ml. min^{-1} 100 g^{-1})	ChBF (ml. min^{-1} 100 g^{-1})	FBP (torr)	$[O_2]_a$ (mM)	PO_2 (torr)	PCO_2 (torr)	pH
	1				4.06	23	38	7.44
○	2	37	1312		5.58	36	44	7.38
	3	45	1042		4.02	23	39	7.44
	1	189	2930		2.50	18	47	7.36
◑	2	137	2249		3.30	21	47	7.37
	3	117	1495		3.08	21	48	7.36
	1	115	1551		1.61	18	42	7.34
●	2	141	2036		1.07	14	40	7.24
	3	100	3626		3.21	26	43	7.30
	1	116	2264	46	3.61	23	42	7.37
▽	2	161	1550	48	1.75	16	35	7.33
	3	150	1897	47	3.08	22	38	7.38
	1	64	1354	37	3.17	25	44	7.38
△	2	89	931	36	2.11	21	42	7.36
	3	62	927	38	3.21	29	46	7.37
	1	258	1330	45	2.77	21	47	7.35
◭	2	374	1411	49	2.14	17	44	7.32
	3	334	1596	47	2.10	18	41	7.37
	1	124	1338	35	2.98	22	48	7.34
▲	2	234	1649	42	1.26	16	39	7.21
	3	148	1721	34	2.56	20	47	7.34
	1	85	1797	29	4.19	27	45	7.36
+	2	96	1725	28	4.24	28	50	7.31
	3	179	2215	32	1.23	16	41	7.33
	1	64	1065	28	5.03	28	38	7.45
*	2	64	919	30	4.59	27	30	7.46
	3	233	1666	41	1.45	18	26	7.23
	1	62	1215	36	4.60	25	49	7.38
◪	2	73	1049	36	3.80	27	46	7.35
	3	162	1173	42	1.41	15	41	7.35
	1	78	1244	40	4.64	25	54	7.32
■	2	141	1418	43	1.80	14	48	7.32
	3	260	1728	51	1.21	12	38	7.26
	1			50	6.62	30	47	7.27
□	2	89	2141	52	5.84	25	42	7.42
	3	303	2551	70	1.34	13	35	7.25

Table 2. *Mathematical functions evaluated for their adequacy in describing the RBF oxygen relation*

Mathematical function	Empiric equation	Estimate for percentage error (%)	Degrees of freedom
Linear function	$f(x) = \mu + \beta \cdot x$	21.0	22
Reciprocal function	$f(x) = \mu + \beta \cdot x^{-1}/x$	19.0	22
Logarithmic function	$f(x) = \mu + \beta \cdot \ln x$	18.3	22
Exponential function	$f(x) = \mu + \beta \cdot e^{x}$	18.6	21

was significantly higher in newborn lambs (Student t test: $P < 0.01$). The postnatal trend in blood flow to both retina and choroid (Fig. 4) was evaluated as follows. The mean value for RBF and ChBF was calculated for each newborn lamb. The Spearman Correlation coefficient (16) determined for the relationship between these mean values for RBF and ChBF, with age, were $+0.17$ ($df = 9$; $P > 0.1$) and -0.62 ($df = 10$; $P < 0.05$), respectively, indicating no relationship between RBF and age, and a significantly decreasing ChBF with postnatal age.

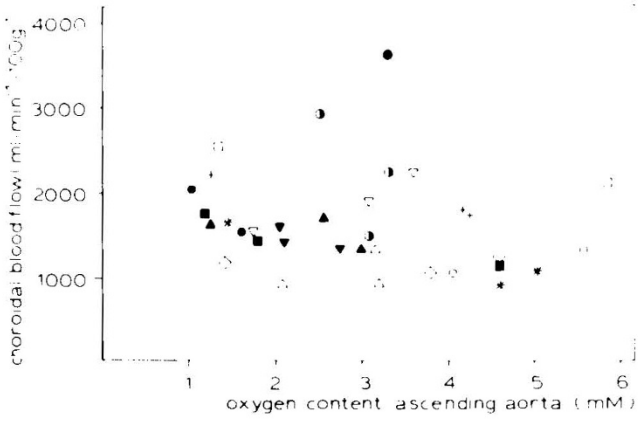

Fig. 2. Relationship between ChBF and $[O_2]_a$ in the ascending aorta. Flow data from different fetuses were identified by using the same 12 symbols as in Figure 1.

DISCUSSION

The curvilinear relationship between fetal RBF and $[O_2]_a$ seemed to be described best by a reciprocal function. Such a relationship would not be surprising because in the same group of animals, the relationship between flow and oxygen in various other central nervous system tissues was also best described by such a function (15). This similarity in flow response to changing levels of $[O_2]_a$ in both fetal retina and central nervous system suggests that a similar mechanism regulates flow to both tissues when the $[O_2]_a$ is altered. In the present study, 82% of the intra-animal variability in RBF could be explained by $1/[O_2]_a$. The

Table 3. *Retinal and choroidal blood flows with the concomitant values for age, systemic BP, PO_2, PCO_2, pH, and $[O_2]_a$ in the ascending aorta of the 12 newborn lambs*

Animal	Post-natal age (days)	Exper-iment no.	RBF (ml. min $^{-1}$ 100 g $^{-1}$)	ChBF (ml. min $^{-1}$ 100 g $^{-1}$)	Mean systemic BP (torr)	$[O_2]_a$ (mM)	PO_2 (torr)	PCO_2 (torr)	pH
1	2	1		2961		4.29	71	43	7.39
2	5	1	86	3966		5.18	65	37	7.42
2	5	2	96	3929		4.82	63	36	7.41
3	19	1	98	2613		5.27	70	37	7.41
4	65	1	53	2839		6.52	69	36	7.40
4	65	2	69	2033		6.65	67	36	7.41
5	100	1	83	2884	79	6.34	65	35	7.47
5	100	2	104	2363	80	6.65	62	35	7.44
5	100	3	89	2930	79	6.70	68	35	7.43
6	148	1	75	1920		6.92	72	37	7.40
6	148	2	46	1985		6.70	73	38	7.39
6	148	3	57	1550		6.88	73	37	7.39
7	75	1	61	3247	108	8.04	64	34	7.37
7	75	2	52	2951	99	7.63	65	35	7.41
7	75	3	77	2867	104	7.81	69	34	7.42
8	149	1	58	2733	90	6.99	73	33	7.39
8	149	2	62	2547	85	6.80	74	31	7.41
8	149	3	90	2658	85	6.61	74	33	7.40
9	226	1	45	2195	101	6.63	71	35	7.42
9	226	3	71	2619		6.33	78	39	7.40
10	13	2	49	3151	75	6.67	61	41	7.40
10	13	3	33	2498	80	6.71	64	37	7.39
11	4	1	36	2130	66	7.12	68	40	7.34
11	4	2	40	3839		6.99	67	37	7.33
11	4	3	41	3924		6.72	67	37	7.34
12	5	1	80	2634	85	6.74	65	42	7.30

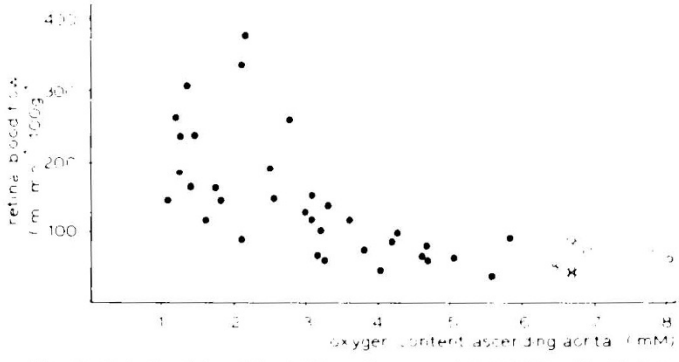

Fig. 3. Relationship of fetal (●) and neonatal (○) RBF with $[O_2]_a$ in the ascending aorta.

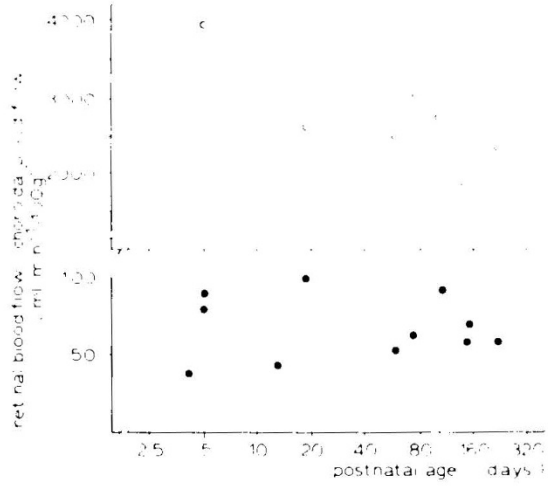

Fig. 4. Relationship of ChBF (○) and RBF (●) with postnatal age. The ChBF decreased significantly with postnatal age.

close relationship between RBF and $1/[O_2]_a$ indicates that the maintenance of oxygen delivery to the retina by way of the retinal vessels is carefully controlled over a wide range of oxygenation. However, it should be emphasized that the microsphere technique as used in this study did not permit assessment of regional flows and oxygen delivery to various areas of the retina. Rather, to have sufficient tissue (and therefore adequate numbers of microspheres) for quantitation, the retinal flow as a whole was studied. It is possible that the reciprocal relationship between RBF and O_2 content described for the retina as a whole, may not be true for a selected area (*i.e.*, the periphery).

To our knowledge, RBF in the fetus has not previously been measured. In adults, it is generally accepted that the arterial PO_2 is an important flow-regulating factor in the retina (6–8). However, it is still obscure whether RBF correlates better with O_2-content or with PO_2 in the supplying artery. In the present study, both $[O_2]_a$ and PaO_2 were altered simultaneously. Thus, it was impossible to determine if one of the two indices for oxygenation correlated with fetal RBF better than the other. Jones *et al.* (10) found that cerebral blood flow in the fetal lamb correlated better with $[O_2]_a$ than with PaO_2. $[O_2]_a$ is of fundamental importance to the organism. Cain (4) found that under circumstances of extreme oxygen deprivation in the adult, whole-body O_2 uptake was determined by $[O_2]_a$ rather than PaO_2. Although there is no such information available for the retina, it is possible that RBF responds directly to changes in $[O_2]_a$. Further investigations are required.

The range of $[O_2]_a$ and PaO_2 in the newborn lambs was too small to permit conclusions about the flow-O_2 relationships in the retinal circulation of the newborn. In the case of both $[O_2]_a$ and PaO_2, however, the neonatal data points appear to follow the trend established by the fetal flow-O_2 relationships in the higher ranges of $[O_2]_a$ and PaO_2 (Figs. 3 and 5). Further experiments covering a wider range of $[O_2]_a$ levels in the neonate are needed

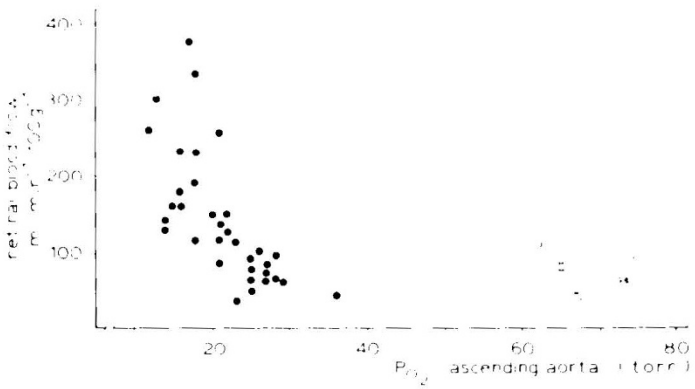

Fig. 5. Relationship of fetal (●) and neonatal (○) RBF with the PO_2 in the ascending aorta.

to determine whether the flow-O_2 relationship is the same during the fetal and newborn periods.

It has been demonstrated that blood flow in the adult retina changes markedly with variations in arterial PCO_2 ($PaCO_2$) (1, 17). In the present study, the range of $PaCO_2$ in both fetal and neonatal lambs was too small to permit a conclusion about the presence of this relationship in these life phases.

Although fetal ChBF was not correlated with $[O_2]_a$, some interesting phenomena were observed in the perinatal period. First, the blood flow to the choroid was significantly higher after birth. Inasmuch as the blood pressure is higher in the newborn (5), this might reflect a lack of autoregulation in this tissue structure, as has been reported for the adult animal by Alm and Bill (1). Second, in the first 5 months after birth, ChBF tends to decrease with age whereas systemic blood pressure has been reported to increase (5, 20). This suggests a delayed development of the ultimate "mature" vascular resistance in the choroidal vascular bed. One might speculate that, in comparison with the mature newborn state, the vascular resistance in the premature choroid is even lower and consequently blood flow is higher at any given perfusion pressure. Such a high flow rate, together with the absence of an O_2-related flow regulating mechanism, would greatly favor the development of high local tissue oxygen tensions in premature babies treated with high environmental oxygen.

Clearly, further studies are needed both in terms of defining regional changes in RBF and ChBF at various levels of oxygenation and also by alteration in O_2 capacity, more sharply delineating the role of PaO_2 *versus* O_2 content in the regulation of RBF.

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APPENDIX

CALCULATION OF THE PERCENTILE ERROR AND THE PERCENTAGE OF INTRA-ANIMAL VARIATION EXPLAINED BY A GIVEN FLOW-OXYGEN MODEL, ANALYZED WITH THE "WEIGHTED LEAST-SQUARES METHOD"

(stochastic variables are underlined)

$$\underline{y}_{ij} = \mu + \alpha_i + \beta \cdot f(\gamma, x_{ij}) + \underline{\varepsilon}_{ij} \quad (i = 1, \cdots, N; j = 1, \cdots, N_i)$$

where N = number of animals; N_i = number of experiments in the i^{th} animal.

$$\sum_{i=1}^{N} \alpha_i = 0$$

where y_{ij} = blood flow measurement in the j^{th} experiment of the i^{th} animal; μ = a constant equal for all animals; α_i = additive effect due to the i^{th} animal; β, γ = regression coefficients assumed to be the same for each animal; x_{ij} = value for $[O_2]_a$ in the j^{th} experiment of the i^{th} animal; $f(\gamma, x_{ij})$ = function of x_{ij} and γ {*e.g.*, $f(\gamma, x_{ij}) = 1/x_{ij}$, or $f(\gamma, x_{ij}) = e^{\gamma x_{ij}}$, or $f(\gamma, x_{ij}) = (\ln x_{ij} + \gamma)/x_{ij}$}; ε_{ij} = error term with expectation zero and standard deviation proportional to the expectation of y_{ij}.

The observed value for y_{ij} and not the expected term of $\underline{y}_{ij}$ is substituted for the error term ε_{ij} in the model above.

In this model, estimates $\hat{\mu}$, $\hat{\alpha}_i$, $\hat{\beta}$, and $\hat{\gamma}$ of, respectively, μ, α_i, β, and γ were determined in such a way that

$$\sum_{i=1}^{N} \sum_{j=1}^{N_i} \left[\frac{y_{ij} - \{\mu + \alpha_i + \beta \cdot f(\gamma, x_{ij})\}}{y_{ij}} \right]^2$$

was minimal.

Let $$S_{min} = \sum_{i=1}^{N} \sum_{j=1}^{N_i} \left[\frac{y_{ij} - [\hat{\mu} + \hat{\alpha}_i + \hat{\beta} \cdot f(\hat{\gamma}, x_{ij})]}{y_{ij}} \right]^2$$

The percentile error was determined as follows:

$$\sqrt{\frac{S_{min}}{d.f.}} \times 100\% \qquad (df = \sum_{i=1}^{N} N_i - p)$$

where $p = \begin{cases} \nearrow & N + 1, \text{ in case of one regression coefficient } (\beta). \\ \searrow & N + 2, \text{ in case of two regression coefficients } (\beta, \gamma). \end{cases}$

If it could be assumed that blood flow was independent of $[O_2]_a$ (thus $\beta = 0$), estimates $\hat{\mu}_i$ for $\mu + \alpha_i$ could be determined in such a way that

$$\sum_{i=1}^{N} \sum_{j=1}^{N_i} \left[\frac{y_{ij} - \{\mu + \alpha_i\}}{y_{ij}} \right]^2$$

was minimal.
Let: $\mu_i = \mu + \alpha_i$, then the function:

$$g(\mu_1, \cdots, \cdots, \mu_N) = \sum_{i=1}^{N} \sum_{j=1}^{N_i} \left[\frac{y_{ij} - \mu_i}{y_{ij}} \right]^2$$

would be minimal when the partial derivatives $\frac{\partial g}{\partial \mu_i} = 0$ for $i = 1, \cdots, \cdots, N$

$$\text{thus} \quad \hat{\mu}_i = \frac{\sum_{j=1}^{N_i} 1/y_{ij}}{\sum_{j=1}^{N_i} (1/y_{ij})^2}$$

$$\text{Let} \quad S = \sum_{i=1}^{N} \sum_{j=1}^{N_i} \left[\frac{y_{ij} - \left[\sum_{j=1}^{N_i} (1/y_{ij}) \Big/ \sum_{j=1}^{N_i} (1/y_{ij})^2 \right]}{y_{ij}} \right]^2$$

Remark $0 \leq S_{min} \leq S$
A measure of the explained variation in blood flow values by a given flow-oxygen function was obtained by

$$100 \left[\frac{S - S_{min}}{S} \right] \%$$

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