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Local Regulation of the Uterine Blood Flow by the Umbilical Circulation (39107)

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(Introduced by Dr. J. E. Kendrick)

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The mechanisms that control the uterine blood flow to the near-term pregnancy are very poorly understood. The vascular bed does not exhibit reactive hyperemia (6) or autoregulation (4). Changes in arterial PO_2 within the physiological range do not appear to affect the resistance of this vascular bed (6), and changes in the pH of arterial blood appear to have minor effects (3). Evidence for the existence of local regulation of placental blood flows was provided by Rankin *et al.* (7, 8), who found that the umbilical and uterine flows are very evenly distributed over the surface of the placenta. Such a distribution would be unlikely to occur by chance alone and is, more probably, an indication that the fetal and maternal placental blood flows are matched in a manner similar to the pulmonary matching of ventilation-perfusion ratios. Should this be the case, we would predict that changes in the flows to a region of one of the placental vascular beds would induce compensatory changes in the other vascular bed such that blood would be shunted to better-perfused regions. An example of this type of response would be a decrease in uterine blood flow following fetal death. In 1971, Raye *et al.* (9) performed a series of experiments on pregnant sheep in which the fetus was killed. They found that the uterine blood flow changed very slowly following fetal death and that within 24 hr the uterine blood flow was at approximately 70% of its control value. In this series it was not possible to determine whether the change in uterine blood flow was a local response or whether the uterine flow was responding to a systematic change in the maternal condition. In an effort to determine whether the uterine blood flow exhibits a local dependence upon the umbilical blood flow, we have performed a series of experiments in which portions of the umbilical vascular bed are occluded. The response of the uterine vascular bed serving these regions is observed and compared to those parts of the uterine vascular bed which were faced with a relatively normal umbilical circulation.

Materials and methods. The experimental series had two parts. In the first part pregnant sheep were used as the experimental animal and a small portion of the umbilical vascular tree was ligated. In the second part the near-term pregnant rabbit was used as the experimental preparation, and one fetus of the litter was killed with the remaining littermates providing control placentas.

Part 1. Sheep series. Crossbred sheep were used at the 125th day of pregnancy. The animals were sedated with intravenous nembutal, and a spinal anesthetic of 2 mg pontocaine was administered. Nembutal sedation was continued as necessary throughout the surgical procedure. The maternal left ventricle was catheterized with a PE200 catheter into which was inserted a soft polyvinyl catheter (i.d. 0.15 mm). This catheter was inserted into the maternal carotid artery and advanced until the tip was in the left ventricle. Subcutaneous xylocaine was applied as a local anesthetic during this procedure. At this time radioactive microspheres labelled with Ce^{141} or $S4^{85}$ were injected into the maternal left ventricular catheter. The injectate consisted of approximately 0.05 mCi of radioactivity embedded on plasticized microspheres (3M Co.) which were 25 μm in diameter. The injectate contained between 1 and 2 million microspheres. The maternal abdomen was then opened through a midline incision, and a small incision was made in the uterine wall. The umbilical blood vessels were detected by manual palpation, and a branch of the umbilical arterial tree was located which had a diameter of approximately 4 mm. This vessel was clamped and tied. The uterine incision was repaired and

the maternal abdomen closed. The carotid catheter was secured in a neck pouch. The sheep was allowed to recover for 24 hr at which time a second injection of microspheres was made into the left ventricular catheter. These spheres were labeled with whichever of the two isotopes not used in the first injection.

The sheep was then killed by intravenous injection of barbiturate followed by potassium chloride. The uterus and contents were removed and the cotyledons were dissected free of the uterine tissue. The cotyledons were cut into 1 g slices and placed into wide counting vials. In no case did the tissue rise more than 1 cm above the bottom of the counting vial. A Ce^{141} and a Sr^{85} standard was prepared. Each standard contained a known number of microspheres of the appropriate isotope. Radioactivity was counted on a Nuclear Chicago 1185 gamma counter. A sample run was prepared which consisted of the two standard vials followed by the vials containing the cotyledonary slices. The spillover of each isotope into the other channel was determined from the information provided from the standard vials, as was the number of counts per min per sphere. The cm of each isotope in each vial and the number of spheres in each piece of cotyledonary tissue were then determined by solving the appropriate algorithm. The number of spheres of each isotope in the cotyledons to which the umbilical flow had been ligated was compared to the number of spheres in those cotyledons that received normal umbilical inflow. The cotyledons that had been subjected to umbilical occlusion were easily identified by their gross appearance. They were edematous and had a brown discoloration. To check that these were indeed the cotyledons in question, a series of three experiments was performed in which a branch of the umbilical artery was ligated and radioactive microspheres were injected into a fetal vein. Twenty-four hours later it was verified that microspheres were present in all cotyledons except those which were grossly identifiable as the cotyledons that had been subjected to umbilical arterial ligation.

Part 2. Rabbit series. The rabbit is multiparous so that if one fetus is killed, then the uterine flow to that placenta can be compared with the uterine flow to the placentas of the remaining living fetuses. Experiments were performed at the 28th day of gestation. The rabbits were anesthetized with intravenous nembutal, and a left ventricular catheter was placed via the carotid artery using a polyvinyl catheter (i.d. 0.5 mm). Radioactive microspheres containing the same isotopes that were used in the sheep series were injected into the maternal left ventricular catheter. Approximately 0.01 mCi were injected in a bolus of approximately 0.1 ml that would contain approximately 200,000 microspheres. The abdomen was then opened and the uterus identified. A fetus in the middle of one of the horns was killed by piercing the adjacent uterus with a 22 gauge needle, which was guided into the fetal chest cavity. The needle was manipulated in such a way as to break the large blood vessels in the fetal thoracic cavity. Placental transfer of barbiturates is rapid, and fetal anesthesia was assured by performing this procedure approximately 1 hr after the induction of maternal anesthesia. The maternal abdomen was then closed and the carotid catheter secured in a neck pouch.

The rabbit was allowed to recover for 24 hr; then a second injection of radioactive microspheres was given via the maternal left ventricle. As with the sheep series, the second injection contained the radioactive label that was not contained in the first injection. The rabbit was then killed by intravenous injection of barbiturate followed by potassium chloride. The uterus and contents were removed, and each individual placenta was removed and placed in a wide counting vial for assay.

Assay was performed in the same manner as that described in the sheep series. The number of microspheres of each type in the placenta serving the dead fetus was compared with the average number of microspheres in the placentas of the two adjacent fetuses.

Results. Part 1. Successful experiments were completed in four sheep. The percentage of the umbilical circulation that was occluded in each placenta was 17.4%, 7.3%, 8.1%, and 5.5% in Sheep 1–4, respectively. Several cotyledons were occluded in each

TABLE I. LOCAL RESPONSE OF THE NEAR-TERM SHEEP PLACENTA 1 DAY AFTER UMBILICAL ARTERIAL LIGATION

Sheep number	Cotyledon number	Cotyledonary weight	Control[a] spheres	Experimental spheres	R_0[b]	R_1	R_1/R_0
1	1	6.87	832	741	0.61	0.47	0.77
	2	3.98	549	607	0.69	0.66	0.96
	3	6.84	1012	854	0.74	0.54	0.73
	4	6.04	859	950	0.72	0.68	0.94
	5	5.55	928	865	0.84	0.67	0.80
	6	3.35	617	556	0.93	0.72	0.77
	7	6.45	970	856	0.76	0.72	0.95
2	1	7.31	1030	711	0.58	0.32	0.55
	2	2.08	375	340	0.75	0.55	0.73
	3	4.80	548	492	0.48	0.34	0.71
3	1	3.05	2883	745	1.68	0.99	0.59
	2	5.03	3071	868	1.40	0.67	0.49
	3	4.90	3709	722	1.34	0.59	0.44
	4	1.66	1535	465	1.63	1.12	0.68
	5	4.27	3004	749	1.25	0.70	0.56
	6	4.56	4028	1173	1.57	1.03	0.65
	7	6.44	4917	1040	1.35	0.64	0.47
4	1	3.02	2513	2552	1.02	0.71	0.69
	2	3.67	2018	1604	0.67	0.37	0.55
	3	2.19	2268	2219	1.00	0.67	0.67
	4	3.68	2906	2020	0.97	0.46	0.47
	5	2.92	1979	1845	0.83	0.53	0.64
	6	2.25	1830	1672	1.00	0.63	0.63
Mean					0.92	0.64	0.67
					$P < 0.01$		

[a] "Experimental" refers to those observations made 1 day after umbilical arterial ligation and "Control" refers to the observations made before umbilical occlusion.

[b] "R" denotes the ratio of spheres/gram in a cotyledon divided by the number of spheres/gram observed in those parts of the placenta to which the umbilical flow was not occluded, before umbilical occlusion (R_0), and 1 day after umbilical occlusion (R_1).

placenta. Data pertaining to the number of occluded cotyledons, the weight of those cotyledons, and the number of spheres observed in each cotyledon before the umbilical flow was occluded and 1 day after the umbilical occlusion, are shown in Table I. Table I also contains the data pertaining to the ratio of the spheres per gram in the cotyledon in question divided by the spheres per gram observed in that part of the placenta to which the umbilical flow was not occluded. These ratios are designated as R_0 for the control ratio and R_1 for the ratio as observed 1 day following umbilical arterial ligation. The response of the cotyledons with umbilical arterial ligation is compared to the response of the nonligated cotyledons by using the ratio R_1/R_0. These data are provided in the last column of Table I. The mean value for R_0 is 0.92 and the mean value for R_1 is 0.64. The upper tail probability of the t value obtained from these data using a paired t test was less than 0.01. The mean value of R_1/R_0 was 0.67, indicating that 24 hr after umbilical occlusion, the uterine flow per gram of placenta was 67% of the value it would have been had the umbilical flow circulation not been occluded.

TABLE II. LOCAL RESPONSE OF THE NEAR-TERM RABBIT PLACENTA 1 DAY AFTER LOSS OF THE UMBILICAL BLOOD FLOW DUE TO FETAL DEATH

Rabbit number	Control		1 day later		R_0[b]	R_1	R_1/R_0
	Experimental[a]	Adjacent	Experimental	Adjacent			
1	678	757	840	819	0.90	0.66	0.73
2	1395	450	576	650	3.10	0.89	0.29
3	399	431	506	603	0.93	0.71	0.76
4	1441	1539	387	1249	0.94	0.31	0.33
5	1357	1526	809	1173	0.89	0.69	0.78
6	956	708	339	369	1.35	0.92	0.68
7	721	748	498	690	0.96	0.72	0.75
8	522	474	680	1062	1.10	0.64	0.58
Mean	934	829	579	827	1.27	0.69	0.61
	NS		$P = 0.02$		$P = 0.02$		

[a] "Experimental" refers to the number of spheres found in the placenta serving the fetus to be killed and "adjacent" refers to the average of the number of spheres in the two adjacent placentas.

[b] "R" refers to the ratio (experimental/adjacent) before (R_0) and 1 day after (R_1) fetal death.

Part 2. Successful experiments were performed upon eight pregnant rabbits. Table II shows the number of spheres that were observed in each of the experimental placentas and the average number of spheres that were observed in the nonexperimental placentas on either side of the experimental placenta. These data are provided for the control in-

jection and for the injection which was made within 24 hr. Two ratios are also defined, R_0 pertaining to the control period and R_1 pertaining to the injection made after 1 day. These ratios are the ratio of the number of spheres in the experimental placenta divided by the average number of spheres in the adjacent placentas. The ratio R_1/R_0 defines the response of the experimental placenta in comparison to the response of the control placenta. The mean of the R_0 values is 1.27, and the mean of the R_1 values is 0.68. These data yield a paired t value for which the upper tailed probability is 0.02. The mean of the R_1/R_0 values is 0.61, which means that 24 hr after fetal death, the uterine flow to that placenta was only 61% of the predicted value. In another series of six rabbits, the second injection was made 5.5 hr after fetal death. In this series we could demonstrate no effect of fetal death on placental blood flow.

Discussion. The radioactive microsphere method is well established for the measurement of regional blood flows, and the applications that have been used here fall well within Buckberg's criteria (2), which specify that for a reasonable degree of statistical accuracy, at least 400 microspheres should be in each piece of tissue. It can be seen in Tables I and II that most of the cotyledons and most of the rabbit placentas contained far more than 400 microspheres.

In the experiments of Raye *et al.* (9), in which the sheep fetuses were killed, the uterine flow declined to approximately 70% of its control value within 24 hr. In the experimental series herein described, we observed that in the sheep the local uterine flow is at 67% of its predicted value 24 hr after the umbilical flow has been ligated. In the rabbit we observed that the local uterine flow is at 61% of its predicted value 24 hr after one of the several fetuses has been killed. It is apparent that these observations are similar to those obtained by Raye *et al.* It is highly probable that the local responses that we observed in this series and the responses of the whole uterus reported by Raye *et al.* pertain to the same phenomenon.

The fact that the depression of the uterine flow seen in our series was only apparent in a relatively small part of the whole uterine vascular bed indicates that the responses were not due to systemic maternal responses but were local responses of the uterine vasculature to the loss of the adjacent umbilical circulation. This local dependence of the uterine blood flow on the umbilical circulation is a relatively slow response, and neither our series nor the series of Raye *et al.* was able to demonstrate significant changes within a few hours of the umbilical occlusion.

The mechanism of the response is not clear at this time. Histological studies performed on the experimental series here described have shown that 24 hr after umbilical occlusion there is evidence of necrosis in the center of the fetal villi. The necrosis is located primarily in the center of the villi and is also diffusely spread throughout the fetal tissue. The uterine flow may therefore be responding to structural changes in the vicinity of the maternal vascular bed. It is also possible that the uterus is responding to a change in the chemical environment of its vasculature. Bell (1) has shown that a living fetus is required for the elaboration of certain vasoactive agents that appear in the uterine venous outflow following uterine arterial constriction. The decline in the uterine blood flow which we have observed here, therefore may be due to the withdrawal of necessary chemical agents which are normally provided by the fetal circulation. The results presented in this series of experiments indicate that, at the local level, the normal near-term uterine blood flow is dependent upon the presence of a functioning umbilical circulation.

Summary. Observations were made of the responses of the uterine blood flow in the near-term pregnancy to occlusion of the umbilical circulation to a few cotyledons of the near-term sheep placenta and in one placenta of the multiparous rabbit pregnancy. It was found that the uterine blood flow declined to 67% of its predicted value 1 day after umbilical ligation in the sheep placenta and to 61% of its predicted value 1 day after the death of one of the fetuses of the rabbit pregnancy. The change in the uterine blood flow in response to the occlusion of the umbilical blood supply to the adjacent area is a local response and is similar in its time course

and magnitude to the response of the whole placenta which has been previously observed by Raye *et al.* (9). This local response of the uterine blood flow is considered to be evidence that the uterine blood flow is in part determined and controlled by the structural or chemical nature of the adjacent fetal compartment.

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1. Bell, C., *Amer. J. Obstet. Gynecol.* **117,** 1088 (1973).
2. Buckberg, G. D., Luck, J. C., Payne, D. B., Hoffman, J. I. E., Archie, J. P., and Fixler, D. E., *J. Appl. Physiol.* **31,** 598 (1971).
3. Buss, D. D., Bisgard, G. E., Rawlings, C. A., and Rankin, J. H. G., *Amer. J. Physiol.* **228,** 1497 (1975).
4. Greis, F. C., Jr., *Amer. J. Obstet. Gynecol.* **96,** 41 (1966).
5. Makowski, E. L., Meschia, G., Droegemueller, W., and Battaglia, F. C., *Amer. J. Obstet. Gynecol.* **101,** 409 (1968).
6. Meschia, G., and Battaglia, F. C., *in* "Foetal and Neonatal Physiology, Proceedings of the Sir Joseph Barcroft Centenary Symposium," p. 272. Cambridge Univ. Press, London/New York (1973).
7. Rankin, J. H. G., Meschia, G., Makowski, E. L., and Battaglia, F. C., *Amer. J. Physiol.* **219,** 9 (1970).
8. Rankin, J. H. G., and Schneider, J. M., *Resp.* (1975), **24,** 373–383.
9. Raye, J. R., Killam, A. P., Battaglia, F. C., Makowski, E. L., and Meschia, G., *Amer. J. Obstet. Gynecol.* **111,** 917 (1971).

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