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Oxygen Consumption in Fetal Rabbit Ductus Arteriosus[1,2] (38590)

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Oxygen is a major stimulus to closure of the ductus arteriosus (1–3). Fay has shown with a double perfusion system that the ductus arteriosus of the guinea pig has a sensitivity to oxygen greater than previously reported (4). Those studies suggest that oxygen triggers contraction in the ductus by a sequence of events, the first of which is interaction with cytochrome a_3 of the oxidative phosphorylation system (5).

In 1972, McMurphy *et al.* studied the effects of gestational age on the *in vitro* contractile response of the fetal lamb ductus arteriosus to oxygen. With increasing gestational age there was an increase in ductal constriction at a given pO_2 and the responses occurred at a lower level of pO_2 (6). If oxygen responsive contractility of the ductus increases with gestational age, and contraction is mediated through the cytochrome system, then it is reasonable to suggest that oxygen consumption of ductus arteriosus tissue would increase with gestational age. This report tests that hypothesis, and demonstrates that oxygen consumption in fetal rabbit ductus arteriosus does increase with gestational age.

Methods. Pregnant rabbits with the gestational age known (25–29.5 days) within 2 hr were sacrificed by a blow to the head and the fetuses were quickly removed via cesarean section and placed into ice water before their first breath and then weighed. Only tissue from fetuses weighing within two standard deviations from the mean were used. The ductus arteriosus and, when studied, descending aorta were dissected out of the tissues and placed in Krebs–Ringer's phosphate buffer with 0.1 g/100 ml glucose at approximately 4°. Care was made in the dissection not to include any other surrounding arterial, adventitial, or pericardial tissue.

The tissues were then minced and placed in Warburg flasks with Krebs–Ringer's phosphate containing 0.1 g/100 ml glucose. The center well of the flask contained 0.2 ml of 5 *N* KOH and 1 cm^2 of filter paper to trap CO_2 produced. The oxygen consumption at 37° was determined on a standard Warburg apparatus, taking measurements every 10 min until a drop in the oxygen consumption occurred. The dry weight of the tissue was determined after dessication to a constant weight.

Dissection of the ductus tissue required fine technique. Therefore, it was necessary firstly to study the time available for dissection and mincing that still gave viable tissues and secondly to determine the minimal amount of tissue that gave reproducible oxygen consumption values. Because of its easy availability, human umbilical artery tissue was used for these studies. Artery tissue was left at 4° for varying times and then minced and the oxygen consumption measured. A steady state of oxygen consumption persisted for 90–100 min after dissection, then there was a sharp drop (Fig. 1). Thus, no rabbit ductal tissue was used in experiments unless the dissection was completed within 40 min of sacrificing the fetus. This allowed several 10 minute interval measurements of reliable oxygen consumption.

The amount of tissue necessary for an acceptable degree of reproducibility was studied also with human umbilical artery tissue of different weights. A tissue dry weight of greater than 15 mg was necessary to obtain reliable data (Fig. 2). This necessitated pooling of rabbit fetal tissue from an entire litter (6–8 fetuses).

Results. The oxygen consumption of the

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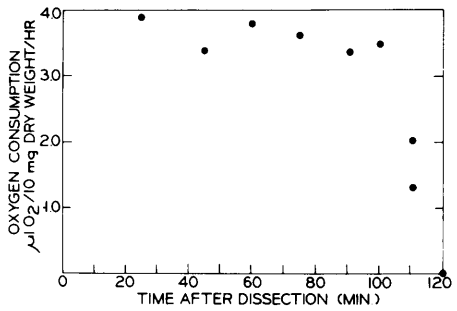

FIG. 1. Effect of time after tissue removal on oxygen consumption. Human umbilical artery of three term neonates was dissected and stored in Krebs-buffer at 4° for various times. Samples were then removed and oxygen consumption was measured as described in the text. Each point represents a different sample from one of the three arteries.

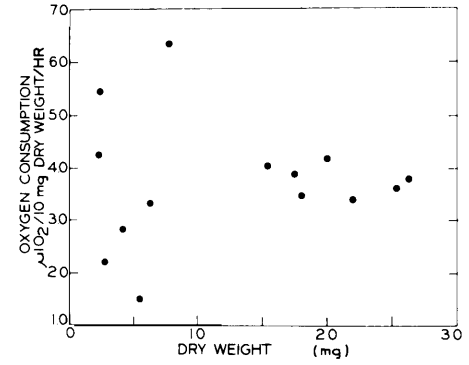

FIG. 2. Oxygen consumption was related to dry weight of tissue studied. Each point represents a different sample of randomly selected amounts of human umbilical artery of three term neonates.

ductus arteriosus tissue from gestational ages of 25.75–29.5 days was measured (Fig. 3). The rabbit fetuses of 25.75–26.5 days had an oxygen consumption of 2.05 ± 0.22 μl oxygen per 10 mg dry wt/hr. The 27-day litter had an oxygen consumption of 3.66 and the 27.5–29.5 day fetuses of 5.69 ± 0.28 (Fig. 3). The difference between the 25.75–26.5 days and the 27.5–29.5 days values was statistically significant ($P < 0.001$).

Fetal rabbit descending aorta was also studied at the same gestational ages. There was no change with gestational age in the oxygen consumption in the descending aorta (2.15 ± 0.23 μl oxygen per 10 mg dry wt/hr).

Cyanide abolished measurable oxygen

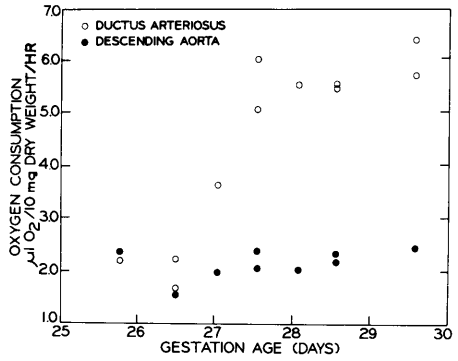

FIG. 3. Effect of gestational age on oxygen consumption. Fetal rabbit ductus arteriosus and descending aorta oxygen consumptions were studied at the gestational ages indicated.

TABLE I. THE DUCTUS ARTERIOSUS AND THE DESCENDING AORTA OF RABBIT FETUSES AT 26.5 AND 27.5 DAYS GESTATION WERE PLACED IN SOLUTIONS OF 1.0 mM CYANIDE AND 15 μg/ml KANAMYCIN WITH 10 μg/ml OF PENICILLIN AND THE OXYGEN CONSUMPTION WAS MEASURED.

Gestational age	Oxygen consumption (μl O_2/10 mg dry wt/hr)			
	Ductus arteriosus		Descending aorta	
	Control	Cyanide	Control	Antibiotics
26.5	1.87	0	2.13	2.30
27.5	5.37	0	2.31	2.16

consumption in fetal rabbit ductus arteriosus. There was no change in umbilical artery oxygen consumption with antibiotics in the solution (Table I).

Discussion. Several reports suggest an increased incidence of the persistent patent ductus arteriosus in the premature neonate (7–12). The reason is uncertain. Frequent hypoxia (10) or the failure of response to oxygen has been implicated. Histological studies in puppies suggest that immature enzyme systems may be involved (13).

Oxygen has a marked constricting effect on isolated ductus arteriosus tissue that is probably separate from the hormonal mechanisms for constriction (2). Oxygen consumption has been abolished by cyanide and constriction was blocked by dinitrophenol in similar *in vitro* systems. In guinea pig ductus arteriosus (4, 5) oxygen consumption

and changes in the oxidation state of cytochrome a_3 as a measure of oxidative phosphorylation preceded contraction, suggesting a close involvement between the cytochrome chain and contraction. Prior to 90 days gestation, the fetal lamb ductus arteriosus responded poorly to oxygen, but from 90 to 150 days gestation, the level of oxygen required for the initial contractile response decreased as the gestational age increased (6). A similar maturation of ductus arteriosus oxygen receptors in human tissue has been suggested (14).

The above observations suggest that oxygen consumption of the ductus arteriosus might vary with gestational age, as was shown in this report. The oxygen consumption of rabbit ductus arteriosus and aorta was relatively low (2–6 μl O_2/10 mg dry wt/hr). However, the reliability of the measurements was assured by completing the dissections in the same time period (35–40 min) (Fig. 1), using 15–20 mg dry wt of tissue (Fig. 2), taking manometric measurements every 10 min, and computing oxygen consumption from a steady linear portion of the gas uptake curve. There was no oxygen consumption in the presence of cyanide, and bacterial contamination of the specimens, if present, was not significant enough to alter oxygen consumption (Table I).

The oxygen consumption of fetal rabbit ductus arteriosus of 25–26 day gestations was significantly lower than that of 27.5 day gestations or greater. This appears to be a property unique to the ductus arteriosus itself, since fetal rabbit aorta tissue had a steady rate of oxygen consumption, regardless of gestational age. There are several possible explanations for the increasing oxygen consumption of ductus arteriosus with gestational age, including an increase in the number of mitochondria, maturation of the cytochrome systems, or specific enzyme activation, but the exact mechanism of the phenomenon requires further studies.

Summary. Oxygen consumption in fetal rabbit ductus arteriosus and descending aorta was studied after human umbilical artery tissue was used to standardize the technique. A steady state of oxygen consumption persisted for 90–100 min after dissection, and a tissue dry weight of greater than 15 mg was necessary to obtain reliable data, necessitating pooling of rabbit fetal tissue from an entire litter. Ductus arteriosus tissue from 25.75–26.5 day gestation had an oxygen consumption of 2.05 $\pm$ 0.22 (4) μl oxygen/10 mg dry wt/hr, and at 27.5–29.5 it was 5.69 $\pm$ 0.28 (7) ($P < 0.001$). There was no change with gestational age in the oxygen consumption in the descending aorta (2.15 $\pm$ 0.23 μl oxygen/10 mg dry wt/hr).

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