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Developmental Changes in Constriction of the Ductus Arteriosus: Responses to Oxygen and Vasoactive Agents in the Isolated Ductus Arteriosus of the Fetal Lamb

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Extract

Development of the constrictor response of the ductus arteriosus to O_2 and selected vasoactive drugs (acetylcholine and bradykinin) was studied in 20 fetal lambs weighing 680–4800 g (90- to 150-day gestation). The isolated ductus arteriosus was perfused *in vitro* with Tyrode's solution at constant flow, temperature (38°), pH (7.3–7.4), and P_{CO_2} (30–40 mm Hg), and the mean pressure difference across the ductus was measured. Ductal resistance was calculated at different levels of P_{O_2} (10–700 mm Hg), raised stepwise to produce dose-response curves. Three young fetuses failed to respond initially to O_2 and in the other 17 the initial response occurred at progressively lower P_{O_2} levels with advancing gestation. The maximal degree of constriction developed showed a progressive increase with advancing gestational age.

At any given P_{O_2}, both acetylcholine and bradykinin produced a further increase in resistance when added to the perfusion solution, but this further increase was independent of age. The level of P_{O_2} at which an initial response occurred was decreased after exposure to acetylcholine but not bradykinin.

Speculation

The ductus arteriosus of the fetal lamb constricts when exposed to oxygen. The initial level of P_{O_2} at which this constriction occurs decreases, and the maximal degree of constriction increases, with advancing gestational age. The poor response to oxygen of the ductus arteriosus in the immature fetus may be the mechanism responsible for the high incidence of patent ductus arteriosus in premature infants. Constriction following oxygen may be augmented by the exposure to acetylcholine or bradykinin.

Introduction

In the normal transition from the fetal to the neonatal circulation, the ductus arteriosus is usually functionally closed within 10–15 hr after birth [18]. It has been shown that O_2 constricts the ductus arteriosus in both fetal and newborn animals of various species, as well as in the human infant [1, 5, 9–11, 13, 15, 16]. In the premature human infant, ductal closure may be delayed even though oxygenation seems adequate [6].

The isolated perfused human fetal ductus arteriosus from the second trimester of pregnancy does not constrict with O_2, indicating that this response develops later during fetal gestation [14]. There is little information regarding the patterns of response of the ductus arteriosus to changes in Po_2, nor is there any evidence to show when the ductal constrictor response to O_2 develops during gestation. The first part of the present study was designed to examine these relations.

Although increased oxygenation does constrict the ductus arteriosus, it is not clear that this is the only mechanism responsible for normal ductal closure. Several studies have shown that various vasoactive agents may cause ductal constriction. Kovalčik [13] showed that epinephrine, acetylcholine, and also bradykinin constricted the isolated lamb ductus arteriosus. Boréus and others found that acetylcholine and epinephrine produced similar constriction in the isolated human ductus arteriosus during both the early and late portions of the second trimester [3, 4, 14]. In addition, recent studies have demonstrated the interaction of O_2 with bradykinin in relation to other adjustments from the fetal to neonatal circulation [7, 8]. Therefore, in the second part of the study, we examined the interrelations between ductal response to O_2 and to acetylcholine and bradykinin.

Materials and Methods

Studies were performed on 20 fetal lambs weighing 680–4800 g, and between 90- to 150-day estimated gestational age [2]. The ewe was anesthetized with low spinal analgesia (20 mg tetracaine HCl). The fetus was then delivered by cesarean section and prevented from breathing by immediately placing a rubber glove over its head. It was killed by rapid exsanguination. Intact specimens of the ductus arteriosus and adjacent pulmonary arteries and aorta were dissected and immersed in Tyrode's solution (NaCl, 8 g; KCl, 0.2 g; $CaCl_2$, 0.2 g; $MgCl_2 \cdot 6H_2O$, 0.1 g; NaH_2CO_3, 1 g; $NaH_2PO_4 \cdot 1H_2O$, 0.05 g; dextrose, 1 g; and distilled water to 1000 ml). They were prepared for perfusion by cannulating the main pulmonary artery (inflow) and descending thoracic aorta (outflow) and securely suturing both right and left pulmonary arteries and the aorta just proximal to the ductus. This preparation was immersed in a constant temperature (38°) 50-ml bath of the Tyrode's solution and perfused by the same solution in a closed system. A diagram of the perfusion apparatus is shown in Figure 1.

Common reservoirs supplied the system with the perfusion solution through which various gas mixtures of O_2, N_2, and 5% CO_2 were bubbled to change the Po_2 over a wide range (10–700 mm Hg) at constant Pco_2 (30–40 mm Hg). Gas sampling was done on the warmed perfusion solution (38°) just before entry into the ductus; pH, Po_2, and Pco_2 were measured using Radiometer electrodes and blood gas meter. A peristaltic pump [19] provided a constant flow at 70–250 ml/min, depending on fetal weight. The pump was calibrated after each study by timed collection of the perfusion solution. A differential strain gauge transducer

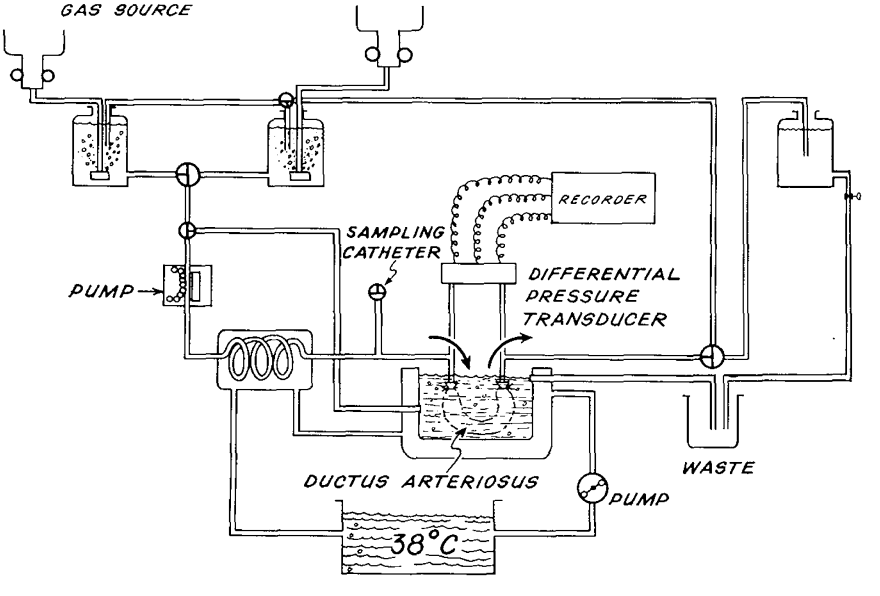

Fig. 1. Diagrammatic representation of the perfusion system used.

[20] continuously monitored proximal (pulmonary arterial), differential, and distal (aortic) mean pressures, which were recorded on a direct-writing polygraph [21]. Ductal resistance ($R_{D,A}$) was calculated by dividing the pressure difference by flow. The ductus arteriosus was initially perfused with the Tyrode's solution at the lowest obtainable Po_2, which was 9–15 mm Hg. The flow rate was adjusted to produce a mean perfusion pressure, proximal to the relaxed ductus arteriosus, of between 35 and 55 mm Hg, depending on fetal weight. The pressure for each perfusion was selected according to our own observations in undisturbed fetal lambs *in utero* at different gestational ages [17]. When the pressure was constant at the required preset level, with constant flow for about 30 min, the preparation was considered to be in a base line state. If any change in the perfusion pressure occurred later at the original base line Po_2 of about 10 mm Hg, the flow rate was adjusted to bring the pressure back to the original level.

Base line mean differential pressure across the ductus arteriosus was 1–3 mm Hg, which gave a calculated base line ductal resistance of 5–50 mm Hg/liter/min, but was generally under 20 mm Hg/liter/min. First, an O_2 dose-response curve was obtained by starting from this base line level at the lowest Po_2 (10 mm Hg) and progressing stepwise up to the highest Po_2 (600–700 mm Hg). Initially, 15–20 mm Hg increments were used until a Po_2 of about 100 mm Hg was reached. Thereafter the Po_2 was raised in steps of 50–100 mm Hg. After any ductal contraction, the Po_2 of the perfusion solution and the proximal pressure were returned to the base line level before proceeding with the next step.

The entire procedure was repeated by starting again at the lowest Po_2 and increasing it stepwise to maximum. In addition, however, after a stabilization period following O_2 exposure, during which either no constriction or a stable O_2 response was elicited at each given Po_2 level, a freshly prepared drug—either acetycholine or bradykinin, was added to produce a known concentration (generally 0.1, 1.0, or 10 μg/ml) in the reservoir for continuous perfusion of the ductus. The drug was then completely washed out of the system from another reservoir and the ductus was relaxed to the base line before proceeding to the next higher step in Po_2 level, with subsequent infusion of the same concentration of drug at the higher level of Po_2. In this way, a second O_2 dose-response curve was also obtained, but followed drug exposure. Continuous exposure to the drug was the only successful means of eliciting a ductal response. There was no ductal constriction following either rapid direct injection of a single bolus of drug into the system just before entry into the ductus or to the addition of the drug directly to the bath containing the ductus.

The response to O_2 before and after drug exposure was evaluated and plotted against the increase in ductal resistance from the base line level. The additional response to the drug at each Po_2 was also plotted.

Determinations were also made, both before and after drug exposure, of the Po_2 level of the first constrictive response and of the maximal increase in ductal resistance from the base line level to the highest Po_2.

Statistical analyses of these observations before and after exposure to either acetylcholine or bradykinin were made and, because of asymmetric distribution of the data, the Wilcoxson signed rank test was used.

Results

Response to O_2

In three fetuses with gestational ages of 103, 120, and 125 days, no increase in pressure difference across the ductus arteriosus occurred when the perfusion solution Po_2 was raised to 650–700 mm Hg.

In the other 17 fetuses, the first increase in pressure difference developed at perfusion solution Po_2 levels between 67 and 700 mm Hg. The relation between gestational age and the level of Po_2 at which the first increase in pressure difference developed is shown in Figure 2. The decrease in the level of Po_2 required to produce the initial constriction was linearly related to advancing gestational age ($P < 0.01$). In the two oldest fetuses of 150-day gestational age, obtained while the ewe was in labor, the constriction first occurred at 67 and 88 mm Hg.

In 5 of these 17 studies, the initial response in 3 was observed only when the Po_2 was raised to 700 mm Hg and, therefore, further stepwise increase was not possible. In two, the study was discontinued for technical reasons after the initial response was obtained. In the remaining 12 studies, the stepwise increase in Po_2 was continued above the level at which the initial constriction was detected. This resulted in a progressive increase in the pressure difference across the ductus arteriosus. In 2 of these 12 studies, rupture of the ductus arteriosus occurred following intense constriction at Po_2 levels of about 300 mm Hg.

The response of the ductus arteriosus to O_2 was slow; the maximal pressure difference at any given

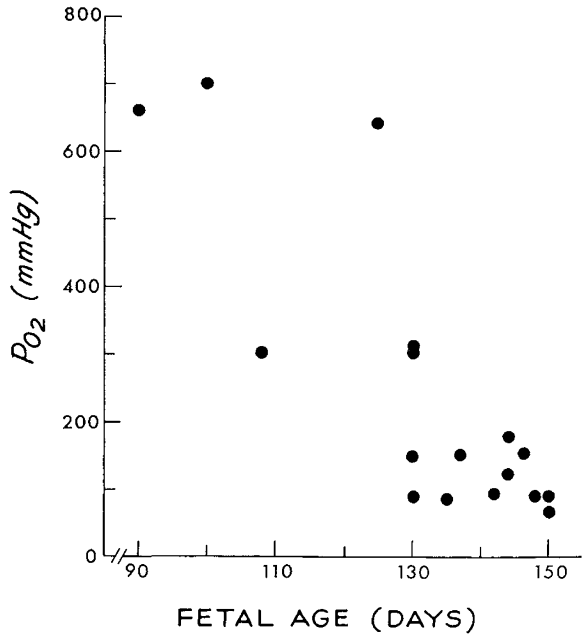

Fig. 2. O_2 level of the initial ductal response to O_2 alone before drug exposure in the isolated perfused ductus arteriosus in fetal lambs of increasing gestational age (90–150 days). Three of the younger fetuses below 125 days did not respond at all and are not shown; four others below 125 days responded only at very high P_{O_2} levels. The older fetuses responded at much lower P_{O_2} levels.

level of P_{O_2} required 5–10 min to develop. Actual recordings from one experiment are shown in Figure 3.

The degree of decrease in ductal diameter with constriction was assessed by calculating the R_{DA} based on pressure-flow relations. The maximal increase in resistance which could be achieved with O_2 was also related to gestational development, as shown in Figure 4. Thus, the amount of ductal contractile response was a function of both O_2 concentration and age. As P_{O_2} was raised, R_{DA} increased and the degree of this response to O_2 increased with age (Fig. 5).

Response to Drugs

The effects of acetylcholine or bradykinin on the ductus arteriosus at different levels of P_{O_2} were examined in 15 of the fetuses. In most instances acetylcholine and bradykinin, when added to the perfusion solution, produced an additional increase in the pressure difference across the ductus arteriosus over and above that produced by O_2 alone at any level of P_{O_2} tested. In one fetus (103 days) neither drug produced constriction; two others (100 and 130 days) responded only to acetylcholine.

The effects of either drug and of O_2 were additive. In eight studies the average maximal increase in R_{DA}

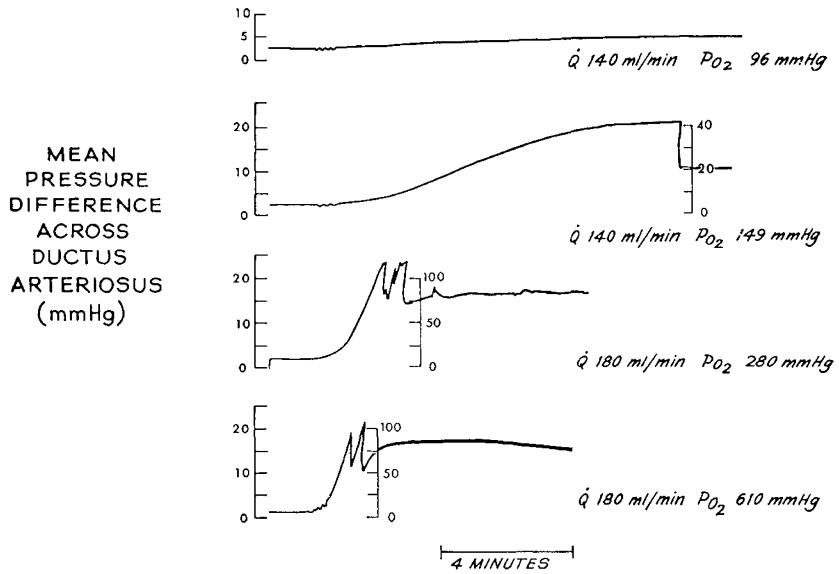

Fig. 3. Actual recordings of O_2-induced contractions in an isolated perfused fetal lamb ductus arteriosus (142 days, 3.5 kg). The mean pressure gradient across the ductus rose progressively from 5 to 90 mm Hg as the P_{O_2} level was increased from 96 to 610 mm Hg. There was no response from a P_{O_2} of 10–96 mm Hg. In between contractions, the P_{O_2} level was reduced to 10 mm Hg so that pressure returned to the base line level of 2.0–2.5 mm Hg. Calculated ductal resistance increased from the base line level of 18 to 500 mm Hg, the maximum value at the highest P_{O_2}. The pH and P_{CO_2} were constant at 7.38 and 40 mm Hg, respectively. In *panels 2, 3,* and *4,* the sensitivity of the recorder was changed when the pressure difference exceeded the original scale.

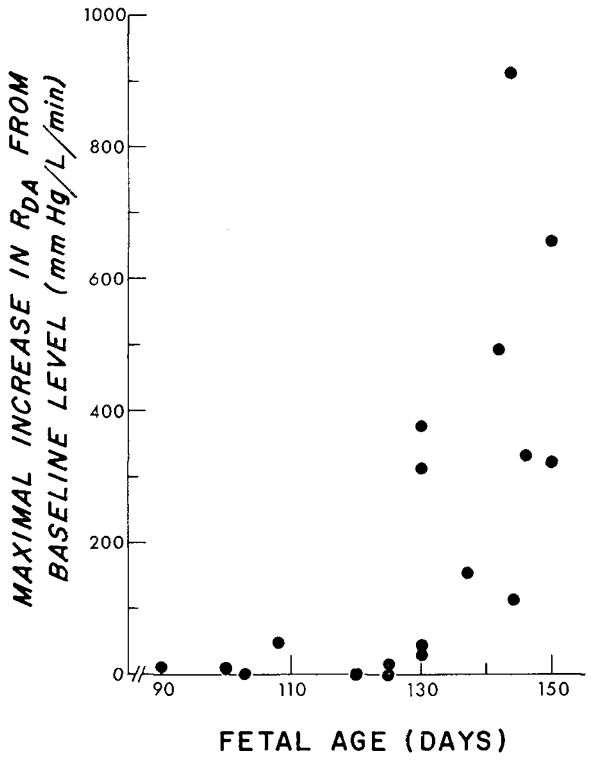

Fig. 4. Maximum ductal response to P_{O_2} levels over 600 mm Hg before drug exposure in the isolated perfused ductus arteriosus in fetal lambs of increasing gestational age (90–150 days). The response is age-related. Three of the younger fetuses (<125 days) did not respond at all. In the four other fetuses < 125 days the increase in maximum R_{DA} from base line relaxation level was considerably less than in fetuses over 130 days old. The zero R_{DA} is the base line resistance, which varied from 7 to 40 mm Hg/liter/min.

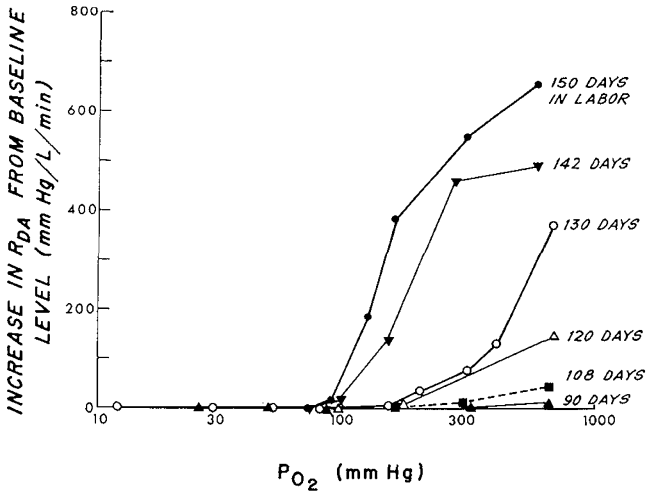

Fig. 5. O_2 dose-response curves from isolated perfused fetal lamb ductus arteriosus of varying gestational ages (90–150 days). The most mature fetus (150 days) was obtained during labor. R_{DA} increased with progressively higher P_{O_2} levels. The earliest responses to O_2 and the greatest degrees of response were seen in the oldest fetuses. The youngest fetuses responded minimally at the highest P_{O_2} level. The zero R_{DA} is the resistance at base line level, which varied from 8 to 40 mm Hg/liter/min.

from base line with O_2 alone was 103 mm Hg/liter/min. After the addition of acetylcholine, at the same P_{O_2} in each instance, the average rose to 290 mm Hg/liter/min ($P < 0.01$). In seven similar studies with bradykinin, the average rose from 237 to 331 mm Hg/liter/min ($P < 0.05$). The ability to respond when exposed to each drug was noted in ductuses from all gestational ages studied. The additional response to bradykinin was noted only when a response to O_2 alone had already been produced. However, addition of acetylcholine produced a constriction at a P_{O_2} level below that at which constriction resulted from O_2 alone. The average P_{O_2} at which constriction was first detected with O_2 alone was 424 mm Hg; following addition of acetylcholine, this fell to 204 mm Hg ($P < 0.05$). An example of the effect of acetylcholine is shown in Figure 6.

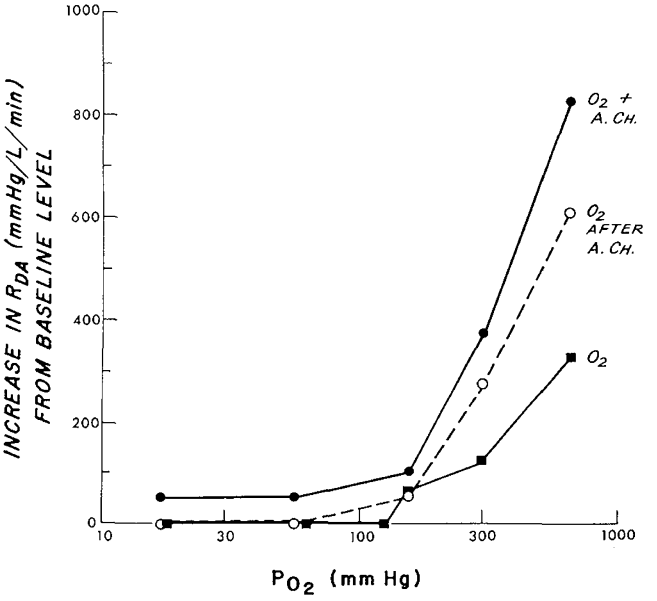

Fig. 6. O_2 response curves for the first O_2 exposure before drugs (■), the second O_2 exposure after previous drug contact (○), and for acetylcholine 10 μg/ml (●) following a stable response of the second O_2 exposure in an isolated perfused fetal lamb ductus arteriosus (146 days, 3.6 kg). The first and second O_2 responses occur initially at the same P_{O_2} (155 mm Hg), but the response of the second O_2 exposure is higher. The initial response to acetylcholine occurs at a much lower P_{O_2} (17 mm Hg) and is greater than the second O_2 response. Zero base line level is 20 mm Hg/liter/min.

Response to O_2 Following Exposure to Acetylcholine and Bradykinin

In 13 of the 20 fetuses, responses to increasing O_2 levels were determined a second time following drug exposure. In three (90, 100, and 103 days), there was no change in initial response; two (120 and 125 days) that had not responded previously to O_2 alone now responded at Po_2 levels of 171 and 630 mm Hg, respectively. Two other young fetuses (108 and 125 days) had previously responded to O_2 (300 and 640 mm Hg), but on second exposure the Po_2 level of the initial response was considerably lower (161 and 290 mm Hg, respectively). In the six older fetuses over 130 days, the Po_2 level of the initial response to O_2 following drug exposure (mean, 149 mm Hg) was unchanged from before (mean, 145 mm Hg). Thus, reduction of the threshold Po_2 level for initial ductal constriction to O_2 after drug exposure occurred only in the group below 130 days.

The maximal degree of constriction that could be obtained with high Po_2 levels following drug exposure was studied in only six fetuses. The average maximal increase in R_{DA} for O_2 exposure prior to drug administration was 208 mm Hg/liter/min; this rose to 289 mm Hg/liter/min on the second exposure to O_2 after drug responses had been measured. Although this showed a trend, it was not statistically significant.

Discussion

Closure of the ductus arteriosus after birth is frequently delayed in premature human infants [6]. Recently, a review of 111 inborn infants weighing under 1750 g revealed that 15.3% had variable degrees of patency of the ductus arteriosus beyond the 1st week of life, confirmed by cardiac catheterization in many instances [12]. The reason for this high incidence has not been evident, but several possibilities are suggested.

A level of Po_2 sufficient to produce ductal constriction may not be achieved if there is inadequate ventilation. Other possibilities include an inadequate amount of ductal smooth muscle to effect constriction, absence of the receptor sites responsive to O_2, or lack of development of a chemical mediator which produces the constrictor response to increased Po_2.

Our present studies provide evidence that, in the fetal lamb, the response of the ductus arteriosus to O_2 is related to gestational age. No constriction was noted in ductuses derived from three young lambs despite a rise in the perfusion solution Po_2 to 650 mm Hg or higher. In those which responded, there was, with advancing gestation, a decrease in the level of Po_2 which produced the initial constriction, ranging from 300–700 mm Hg in the 90- to 110-day fetuses to about 70–90 mm Hg in the term fetuses. Although responses of the isolated perfused ductus arteriosus cannot be compared with those *in vivo*, this latter Po_2 is achieved soon after birth in most premature as well as mature infants. Thus, the high incidence of persistent patency of the ductus arteriosus in premature human infants could be related to a lack of responsiveness of the ductus arteriosus to O_2 at an early gestational age.

We found, too, that the maximal degree of constriction attainable by increasing Po_2 was also related to gestational age. Little or no constriction was obtained in 90- to 125-day fetuses, whereas after 130 days there was a sharp increase in the amount of constriction. Assali *et al.* [1] have shown that in the lamb near term there was a linear relation between ductal flow and Po_2 levels of 50–700 mm Hg. However, we found a curvilinear type of response, with complete constriction and rupture of the ductus, in two instances at a Po_2 level of 300 mm Hg.

It is important to realize that in the type of preparation used in this study, the ductus might have been held open by the high perfusion pressures which developed when constriction occurred. Since the initial pressures and flows were similar to those estimated to be present in the living fetus *in utero*, and since flow was maintained constant, increasing ductal constriction resulted in higher perfusion pressures. It is quite possible that complete closure might have been accomplished if intraluminal pressures were not as high. In the younger fetuses, since high intraluminal pressure did not develop, this was not an important consideration in the failure to demonstrate effective constriction in response to O_2.

The technique may be criticized for its relative insensitivity to demonstrate small changes in muscle length. On the basis of the Poiseuille equation, the pressure difference measured across the ductus with constant laminar flow is inversely related to the fourth power of the radius. Thus, the same change in muscle length would produce a greater change in ductal resistance and pressure differential in a ductus with a smaller resting radius than with a larger one. Therefore, the method that we used would be more sensitive for showing constriction in the younger fetus with a ductus arteriosus of small diameter than in the older fetus. The changes in ductal resistance which we observed in the larger ductuses at later gestation, therefore, indicate considerable degrees of muscular response to O_2 and reduction in lumen diameter.

The direct measurement of changes in muscle ten-

sion is more sensitive than the method that we used and, in preliminary studies with ductal strips or rings suspended in a bath, small degrees of response to O_2 have been observed at gestational ages below 90–100 days.

Acetylcholine and bradykinin each produced an increase in the degree of constriction of the ductus occurring at different Po_2 levels. We did not examine the effect of acetylcholine and bradykinin together, so it is not known whether they share a common pathway in their action. However, an important difference in the effect of acetylcholine and bradykinin was observed. The initial response to bradykinin occurred at the same Po_2 level at which any response to O_2 was first noted; with acetylcholine, the constriction was observed at a lower level of Po_2 than that produced with O_2 alone. This difference in the effect of the two drugs is unexplained, although the mechanism of action of acetylcholine may be independent of O_2, but that for bradykinin may be operative only in the presence of high concentrations of O_2.

Following exposure to both bradykinin and acetylcholine, the ductus arteriosus of the younger fetuses responded initially to a lower Po_2 than before exposure to the drugs. This effect persisted for at least several hours after the drug had been completely washed out of the bath. It could indicate some long term attachment of the drug in amounts which were ineffective alone in producing constriction, or there could be some alterations in the receptors responding to O_2.

These studies suggest that delayed closure of the ductus arteriosus in premature infants is related to an ineffective constriction of their ductuses in response to increases of Po_2. It has not, however, resolved the question as to whether this is due to an inadequate muscular growth or to lack of development of adequate receptor function. The fact that acetylcholine lowers the Po_2 at which ductus arteriosus constriction occurs in the isolated premature lamb ductus suggests the possibility that it could be useful in stimulating such constriction in the premature human infant when O_2 alone is ineffective.

Summary

Constriction of the isolated perfused ductus arteriosus in fetal lambs is produced by increasing the Po_2 of the perfusing solution. The Po_2 level at which constriction is first produced is age-dependent, the ductus from the older fetuses responding at a significantly lower Po_2 level than that from the younger fetuses. Likewise, the maximal constriction developed at high Po_2 (600 mm Hg) is age-dependent, with the ductus arteriosus from older fetuses constricting more than those from younger fetuses.

Both acetylcholine and bradykinin were able to increase the amount of constriction of the ductus arteriosus at any level of Po_2 irrespective of the age of the fetus.

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