The Pharmacology of the Ductus Arteriosus

GORDON C. S. SMITH[a]

Laboratory for Pregnancy and Newborn Research, Department of Physiology, College of Veterinary Medicine, Cornell University, Ithaca, New York

[a] Address for correspondence: Gordon C. S. Smith, Laboratory for Pregnancy and Newborn Research, Department of Physiology, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853-6401. E-mail: gcs4@cornell.edu.

I. Introduction

The ductus arteriosus is a shunt blood vessel of fetal life; it extends between the pulmonary artery and the aorta. It shunts deoxygenated blood from the main pulmonary artery to the descending aorta. Over half of the blood flow in the descending aorta is diverted to the umbilico-placental circulation (Heymann and Rudolph, 1975), where gaseous exchange takes place. The timing of closure of the ductus after birth varies between species (Heymann and Rudolph, 1975), but it is usually complete within 48 h in humans (Drayton and Skidmore, 1987). Ductal patency in utero is an active state principally maintained by the potent dilator effect of prostaglandins (PGs)[b] (Coceani and Olley, 1988). Closure at birth is because of contraction of its smooth muscle. This is secondary to withdrawal of dilation and active stimulation of contraction, particularly by increased oxygen tension (Coceani and Olley, 1988).

The control of the ductus is clinically important in a number of areas. Contraction of the ductus, with or without fetal heart failure, is a recognized side effect of administration of prostaglandin H synthase (PGHS) inhibitors to the mother (Van den Veyver and Moise, 1993). Failure of ductal closure after birth is a common complication of premature delivery, and, conversely, in some forms of congenital heart disease, survival of the neonate is dependent on persistent patency of the ductus (see Gersony, 1986 for review). Understanding the role of PGs in the control of the ductus led directly to therapies in the human neonate, specifically, indomethacin to close the ductus and E series PGs to maintain its patency.

This review seeks to summarize the current state of knowledge of the factors that maintain ductal patency in utero and promote ductal contraction after birth. It also seeks to identify potential novel therapeutic strategies for avoiding ductal contraction as a side effect of maternal anti-PG therapy and for the safer and more effective manipulation of ductal patency in the human neonate.

II. Physiological Roles of the Ductus Arteriosus

A. The Fetal Circulation

Flow across the ductus is determined by the difference in pressure between the two vessels it connects, the pulmonary artery and the aorta. In fetal life, pulmonary artery pressure is high and aortic pressure is low (the latter because of the presence of the low resistance um-

[b] Abbreviations: 4-AP, 4-aminopyridine; AA, arachidonic acid; ABT, 1-aminobenztriazole; AC, adenylate cyclase; AH 13205, ($\pm$)-trans -2- [4- (1-hyroxyphexyl) phenyl] -5-oxocylopentaneheptanoic acid; ATP, adenosine triphosphate; BW245C, 5- (6-carbohexyl) -1- (3-cyclohexyl -3- hydroxypropylamino) hydantoin; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; CO, carbon monoxide; DuP697, 5-bromo-2[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene; eNOS, endothelial nitric oxide synthase; ET, endothelin; GR63799X, [1R-[1a(Z),2b(R*), 3a]]-4-(benzoylamino) phenyl 7-[3-hydroxy- 2 (2-hydroxy- 3- phenoxypropoxy)- 5- oxocyclopentyl]- 4- heptenoate; iNOS, inducible nitric oxide synthase; mRNA, messenger ribonucleic acid; NO, nitric oxide; NOS, nitric oxide synthase; PDA, patent ductus arteriosus; PG, prostaglandin; PGDH, prostaglandin dehydrogenase; PGHS, prostaglandin H synthase; SNP, sodium nitroprusside; Tx, thromboxane; U46619, 5-heptenoic acid, 7-[6-(3-hydroxy-1-octenyl)-2-oxabicyclo[2.2.1]hept-5-yl-,[1R-[1α,4α,5β,(Z),6α,(1E, 3S*)]]-; ZnPP, zinc protoporphyrin IX.

bilico-placental circulation), therefore the flow is right to left (Anderson et al., 1981). The right ventricle pumps approximately two-thirds of the combined ventricular output in the fetal lamb, and 90% of this is shunted from the main pulmonary artery to the descending aorta through the ductus arteriosus (see Heymann and Rudolph, 1975). There is no left to right shunt in the normal lamb fetus (Teitel et al., 1987).

B. The Neonatal Transitional Circulation

After birth, the pressure gradient across the ductus is reversed. Pulmonary arterial pressure falls after ventilation of the lungs with air and aortic pressure rises because of the loss of the low resistance umbilico-placental circulation (Teitel et al., 1987). In the neonate, flow across the ductus is reversed within minutes after birth (Dawes et al., 1955; Drayton and Skidmore, 1987): i.e., from the aorta to the pulmonary artery. It is a common misconception that closure of the ductus is one of the factors that increases pulmonary blood flow in the neonate. Because the shunt across the ductus in the neonate is left to right, ductal patency increases pulmonary blood flow; ligation of the ductus of the term neonatal lamb decreases pulmonary blood flow (Dawes et al., 1955).

It has been proposed that the left to right shunt of the ductus in the neonate may have an important physiological role in adaptation after birth (Dawes et al., 1955). The magnitude of the shunt in the neonatal preterm lamb varies directly with arterial oxygen tension (Clyman et al., 1987), and ligation of the ductus in the term neonatal lamb decreases arterial oxygen tension (Dawes, et al., 1955). A similar improvement in oxygenation can be demonstrated by creating an artificial ductus arteriosus (with left to right shunt) in adult animals; patency of this artificial vessel increases arterial oxygen tension in the face of an experimentally induced pulmonary arterio-venous shunt (Born et al., 1955). Therefore, the physiological patency of the ductus in the term neonate with a left to right shunt acts to improve arterial oxygen tension in the immediate neonatal period when the lungs are not fully expanded (Dawes et al., 1955; Born et al., 1955).

C. Closure of the Ductus Arteriosus

Initial closure of the ductus is mediated by contraction of its thick muscular wall. In some species, closure is complete in the first few hours after birth, e.g. mice (Tada and Kishimoto, 1990), rats (Jarkovska et al., 1992), and rabbits (Momma et al., 1980); in others, however, it occurs in the first 1 to 2 days of life, e.g. lambs (Dawes et al., 1955), guinea pigs (Fay and Cooke, 1972), and humans (Drayton and Skidmore, 1987). After functional closure, the ductus remodels and closure is permanent. A remnant of the ductus is evident in the adult, the *ligamentum arteriosum*, which is formed by fibrosis of the closed neonatal vessel.

III. Factors Maintaining Ductal Patency In Utero

The original view of the ductus was that it represented a relatively passive structure in utero that was actively stimulated to contract after delivery (Kennedy and Clark, 1942). It has become clear, however, that patency of the ductus in utero is an active state. That is, it has intrinsic tone, or is tonically stimulated to contract, and these procontractile mechanisms are tonically inhibited by vasodilators. Probably the most important dilator system identified so far is prostaglandin E_2 (PGE_2), which has a profound inhibitory effect on ductal smooth muscle. However, several accessory dilator systems have also been identified.

Increasing oxygen tension has a profound effect on the ductus to promote contraction, both directly and by modulating its response to vasodilators and vasoconstrictors (Smith and McGrath, 1991, 1993). Although the relatively low oxygen tension to which the fetal ductus is exposed helps maintain patency, the effects of oxygen are discussed in section IV in the context of rising oxygen tension promoting contraction.

A. Prostaglandins

1. Dilator effects of prostaglandins. In 1973, Coceani and Olley demonstrated that prostaglandin E_1 (PGE_1) and PGE_2 relaxed the isolated lamb ductus arteriosus. Several studies have compared the relative potencies of PGs in dilating the ductus in a range of species (rats, rabbits, pigs, and sheep), and PGE_1 and PGE_2 have been found, uniformly, to be the most potent, causing ductal relaxation in the picomolar and low nanomolar range (Coceani et al., 1975, 1978b, 1980; Sharpe and Larsson, 1975; Starling et al., 1976; Clyman et al., 1977, 1978c,d; Momma et al., 1980; Friedman et al., 1983; Sideris et al., 1985; Smith et al., 1994). 6-keto-PGE_1 is almost as potent as PGE_2 and, theoretically, could be formed from PGI_2; however, the enzymes required for this have not been demonstrated in the lamb ductus (Coceani et al., 1980). The only mammalian species studied where PGs have not been shown to exert a significant dilator effect is the guinea pig (Bodach et al., 1980).

2. Prostanoid receptors and signal transduction. The pharmacological classification of prostanoid (P) receptors has been reviewed (Coleman et al., 1994b). Each receptor is named after the native PG that is its most potent agonist [i.e., EP for PGE_1 and PGE_2, IP for PGI_2, DP for PGD_2, FP for $PGF_{2\alpha}$ and TP for thromboxane A_2(TxA_2)]. There are at least four subtypes of EP receptors encoded by separate genes, and there are variable numbers of isoforms (depending on the species) of EP_3 receptors formed by alternative messenger ribonucleic acid (mRNA) splicing from a single gene. The dilator effect of PGE_2 on the rabbit ductus was mediated by the prostanoid EP_4 receptor subtype (Smith et al., 1994); this receptor also mediated dilation of porcine venous smooth muscle by PGE_2 in the picomolar and low nano-

molar range of concentrations (Coleman et al., 1994a). The gene encoding the EP_4 receptor has been cloned and sequenced, and, like the other prostanoid receptors, is a member of the superfamily of G-protein coupled receptors with seven transmembrane domains (Pierce et al., 1995). Normal ductal closure fails to take place in mice lacking the EP_4 receptor gene, confirming that this receptor mediates the physiological effects of PGE_2 on the mouse ductus (Nguyen et al, 1997). When the cloned human and murine genes were transfected into mammalian cells, EP_4 receptors were found to be coupled positively to adenylate cyclase (AC) (Honda et al., 1993; Bastien et al., 1994; Nishigaki et al., 1995). Consistent with this (although the EP receptor subtype mediating the dilator effect of PGE_2 on the lamb ductus has not been confirmed), PGE_2 increased the intracellular concentration of cyclic adenosine monophosphate (cAMP) in the lamb ductus arteriosus (Walsh and Mentzer, 1987). Similarly, the effects of PGE_2 on the staphylococcal α-toxin permeabilized rabbit ductus were potentiated by a phosphodiesterase inhibitor and were identical with forskolin (a direct activator of AC) and exogenous cAMP (Crichton et al., 1997). The cellular mechanism of action of PGE_2 (presumably acting via cAMP) has been at least partially elucidated in the α-toxin permeabilized rabbit ductus, where it was found to inhibit the sensitivity of the contractile proteins to calcium (Crichton et al., 1997).

In the isolated lamb ductus arteriosus precontracted with indomethacin, PGI_2 was about 1000 times less potent than PGE_2 (Coceani et al., 1978b; Clyman et al., 1978c) The relatively low potency of PGI_2 led some authors to dismiss it as having little or no role in the physiological control of ductal patency (Clyman, 1987; Coceani and Olley, 1988). However, the fetal rabbit ductus has IP receptors (Smith et al., 1994). Furthermore, the stable IP receptor agonist, cicaprost, was only 20 to 100 times less potent than PGE_2 (Smith and McGrath, 1994; Smith et al., 1994), and the maximal dilator effect of cicaprost was actually greater than PGE_2 in the isolated rabbit ductus (Smith and McGrath, 1993). These findings suggest a role for PGI_2 in maintaining ductal patency. The cloned human IP receptor is also positively coupled to AC (Boie et al., 1994).

The two other prostanoid receptors that generally mediate relaxation of smooth muscle are the DP and the EP_2 receptors (Coleman et al., 1994b). Although the rabbit ductus also relaxed in response to the DP receptor agonist, BW245C (5-(6-carbohexyl)-1-(3-cyclohexyl -3-hydroxypropylamino) hydantoin), these effects were because of weak agonism of the EP_4 receptor (Smith et al., 1994). The rabbit ductus was insensitive to the selective EP_2 agonist AH 13205 (($\pm$)-trans-2-[4-(1-hyroxyphexyl) phenyl]-5-oxocylopentaneheptanoic acid) even in high micromolar concentrations (Smith et al., 1994). Therefore, the rabbit ductus appears to lack DP and EP_2 receptors.

3. Local sources of prostaglandins. The ductus is exposed to both locally released and circulating PGs (Clyman, 1987). The isolated ductus synthesizes a range of PGs. PGI_2 was the main product of arachidonic acid (AA) in both the ovine and bovine ductus, but it also formed small amounts of PGE_2, $PGF_{2\alpha}$, and PGD_2—all about 10% the level of PGI_2 synthesis (Terragno et al., 1977; Pace-Asciak and Rangaraj, 1977, 1978 and 1983; Skidgell et al., 1984); this adds further support for a physiological role for PGI_2 in the control of the ductus. It has been suggested that PGE_2 is formed by the degradation of PGH_2 in the lamb ductus and not through an enzymatic pathway (Needleman et al., 1981; Skidgell et al., 1984). However, the stimulatory effect of reduced glutathione on PGE_2 release has been interpreted as indicating the presence of PGE_2 isomerase (Coceani et al., 1986). The control of ductal PG synthesis in relation to birth is discussed in section IV and the effect of gestational age is discussed in section V.A.

The cellular sources of PGs in the ductus have been partially elucidated. In the ductus from fetal dogs and the second trimester human fetus, PGI_2 synthase was located (by immunohistochemistry) both in endothelial cells and medial smooth muscle cells (de Reeder et al., 1989; Slomp et al., 1992), whereas in the fetal aorta, the enzyme was largely confined to the endothelium (de Reeder et al., 1989). Similarly, the release of PGE_2 (expressed per unit weight of tissue) from preparations of media alone either equaled or exceeded preparations of media and intima, implying that the media may be the major location of local synthesis of PGE_2 in the isolated lamb ductus (Coceani et al., 1986).

Prostaglandin dehydrogenase (PGDH) activity has been demonstrated in the ovine (Pace-Asciak and Rangaraj, 1978), but not the canine, ductus (de Reeder et al., 1989).

4. Circulating prostaglandins. The ductus is also exposed to circulating PGE_2, and it has been suggested that circulating PGE_2 is more important in the control of the vessel than locally released PGE_2 (Clyman, 1987). Circulating concentrations of PGE_2 increased toward term and were approximately 1 to 2 nM in the late gestation fetal lamb (Clyman et al., 1980b) which is close to causing maximal relaxation of the isolated ductus (Coceani et al., 1975; Smith et al., 1994). The placenta is thought to be the major source of circulating PGE_2 in the lamb fetus (see Thorburn, 1992). Because the lungs are the major site of PG catabolism (Tsai and Brown, 1987) and pulmonary blood flow is only 7% of combined ventricular output in the lamb fetus (Heymann and Rudolph, 1975), the high circulating concentrations of PGE_2 are probably also related to reduced catabolism.

5. Effects of prostaglandin H synthase inhibition in vivo and in vitro. Soon after the initial description of the dilator effect of PGE_2, it was demonstrated that indomethacin, a PGHS inhibitor, contracted the rat ductus in vivo (Sharpe et al., 1974) and the lamb ductus in vitro

(Coceani et al., 1975), which suggested that (a) the net effect of endogenous PGs on the ductus was dilator and (b) that the ductus had intrinsic tone in utero that was being tonically inhibited by PGs. The effect of indomethacin is likely to have been because of its inhibitory effect on PGHS because a range of structurally diverse PGHS inhibitors contracted the rat ductus (Momma et al., 1984). Indomethacin did not affect the ductus arteriosus of fetal mice lacking the prostanoid EP_4 receptor gene, which indicates that the primary mechanism by which indomethacin contracts the vessel in vivo is eliminating the dilator effect of PGE_2 (Nguyen et al, 1997).

The contractile response of the isolated ductus to indomethacin has been assumed to be due primarily to the elimination of the dilator effect of PGE_2 (Clyman, 1987; Coceani and Olley, 1988) because it is the most potent prostanoid dilator of the vessel (see Section III.A.I). However, in the rabbit ductus, indomethacin decreased the maximum response of the vessel to a nonprostanoid dilator (cromakalim) but increased its maximum response to cicaprost, which implied the presence of a tonic dilator effect of PGI_2 (see Smith and McGrath, 1994). Establishing the relative roles of the loss of PGE_2 and PGI_2 in mediating the effects of PGHS inhibitors on the isolated ductus will require the development of potent selective EP_4 and IP receptor antagonists (Smith et al., 1994).

6. Relative roles of local and circulating prostaglandin in fetal life. Early work on the effects of PGs and PGHS inhibitors led to the conclusion that circulating PGs were primarily responsible for maintaining ductal patency in utero (Clyman, 1987). The findings that led to this conclusion were made in the isolated lamb ductus exposed to fetal oxygen tension, where (a) indomethacin had no contractile effect and (b) there was minimal release of PGE_2 (Clyman et al., 1980a). However, the contractile response to indomethacin is not purely an index of the dilator effect of locally released PGs (Smith, 1997). The contraction elicited by indomethacin is also determined by the degree of spontaneous tone in the ductus. The ductus may be profoundly inhibited by locally released PGs, but when there is no spontaneous tone present, it will not contract in response to indomethacin. The lack of a contractile response to indomethacin of the isolated ductus exposed to fetal oxygen tension may simply reflect low levels of spontaneous tone (Smith, 1997).

This hypothesis was tested by examining the effect of indomethacin on induced tone of the ductus exposed to fetal and neonatal oxygen tension. It was found that indomethacin induced a ten-fold increase in the sensitivity of the neonatal rabbit ductus to norepinephrine exposed to both fetal and neonatal oxygen tension (Smith and McGrath, 1988), implying a similar degree of tonic inhibition by locally produced PG across this range of oxygen tension. Furthermore, varying the spontaneous tone of the isolated fetal rabbit ductus by altering

the degree of passive stretch profoundly affected the magnitude of the response to indomethacin (Smith, 1997). Taken together, these observations indicate that the magnitude of the contraction induced by indomethacin in the isolated rabbit ductus is an unreliable index of the dilator effect of locally released PGs and that a lack of contractile response to indomethacin in the isolated ductus exposed to fetal oxygen tension does not exclude a role for locally released PGs in maintaining patency of the ductus arteriosus in fetal life. This conclusion is underscored by the fact that, although some studies failed to demonstrate a contractile effect of indomethacin on the isolated lamb ductus exposed to fetal oxygen tension (Clyman et al., 1980a), another laboratory has consistently observed a contractile effect in response to this drug under virtually identical conditions (Coceani et al., 1975, 1986).

7. Prostaglandin H synthase isoforms. There are two isoforms of the enzyme PGHS: a constitutive isoform (PGHS-1) and an inducible isoform (PGHS-2) (see Frolich, 1997). There is a recent preliminary report that the fetal ductus primarily expresses PGHS-1, whereas the neonatal vessel primarily expresses PGHS-2 (Guerguerian et al., 1997). The isoforms present in the placenta, the major source of circulating PGE_2 in the fetus (see Section III.A.4.), vary with gestational age. In the preterm sheep, PGHS-1 predominates, whereas with advancing gestation there is induction of PGHS-2, which is correlated with increased placental PG synthesis (Wimsatt et al., 1993). In the preterm fetal ductus, therefore, both locally released and circulating PGE_2 may be produced by PGHS-1. In the term fetal ductus, locally produced PGE_2 is still formed by PGHS-1 whereas PGHS-2 may be the predominant source of circulating PGE_2.

A selective PGHS-2 inhibitor (DuP697 (5-bromo-2[4-fluorophenyl]-3-[4-methylsulfonylphenyl]-thiophene)) had no effect on ductal patency of the late gestation lamb fetus in utero (Guerguerian et al., 1997). Similarly, DuP697 had no effect on ductal patency or circulating levels of PGE_2 in the neonatal lamb, whereas indomethacin caused contraction of the neonatal ductus and decreased circulating concentrations of PGE_2 (Guerguerian et al., 1997). However, the selectivity of DuP697 for PGHS-2 over PGHS-1 is only about thirty-fold (Riendeau et al., 1997). Consequently, it could not block PGHS-2 completely without also affecting PGHS-1, and, conversely, at a concentration where it had no effect on PGHS-1, it would be unlikely to block fully the effects of PGHS-2. Furthermore, the complete lack of effect of DuP697 on circulating concentrations of PGE_2 in either the fetus or neonate raises the possibility that the drug failed to access the intracellular site of PGHS-2. There are recent reports of PGHS-2 inhibitors that are over a thousand-fold more active at PGHS-2 compared with PGHS-1 (Riendeau et al., 1997). The relative roles of PGHS-1 and PGHS-2 in controlling ductal patency in the fetus and neonate should be clarified by investiga-

tions with these drugs. It may be that activity of either isoform of PGHS is on its own sufficient to maintain ductal patency, as mice lacking either PGHS-1 or PGHS-2 survive the immediate neonatal period (Dinchuk et al., 1995; Langenbach et al., 1995), whereas mice lacking the receptor mediating the dilator effects of PGs die in early neonatal life from patent ductus arteriosis (Nguyen et al., 1997).

B. Nitric Oxide

Both sodium nitroprusside (SNP) and glyceryl trinitrate dilated the lamb ductus in vivo (Walsh et al., 1988) and the lamb and rabbit ductus in vitro (Walsh and Mentzer, 1987; Smith and McGrath, 1993). These agents are nitric oxide (NO) donors, and they increased the intracellular concentrations of cAMP and cyclic guanosine monophosphate (cGMP) in the lamb ductus (Walsh and Mentzer, 1987; Coceani et al., 1996a). Inhibitors of nitric oxide synthase (NOS) contracted the ductus both in vitro (Coceani et al., 1994b) and in vivo (Fox et al., 1996), and contraction of the isolated lamb ductus was associated with decreased intracellular concentrations of cGMP (Coceani et al., 1996a). Removing the luminal endothelium of the ductus decreased, but did not abolish, the contractile response to a NOS inhibitor (Coceani et al., 1994b; Clyman et al., 1997b), implying an extraluminal source of NOS. Immunohistochemistry localized endothelial nitric oxide synthase (eNOS) to the luminal endothelium (Fox et al., 1996) and the vasa vasorum endothelium (Clyman et al., 1997b). The presence of mRNA encoding the inducible nitric oxide synthase (iNOS) gene in a homogenate of the heart and great vessels from the fetal rat has been demonstrated (Bustamante et al., 1996), and immunohistochemistry of the fetal lamb ductus has located this to the luminal endothelium (Clyman et al., 1997b). Oral administration of a selective iNOS inhibitor (L-N^G-1[1-aminomethyl] lysine) to pregnant rats caused contraction of the fetal rat aorta, pulmonary artery, and ductus arteriosus in vivo in a dose-dependent manner, and this could be reversed with SNP (Bustamante et al., 1996).

However, in vitro studies have demonstrated that in the indomethacin-treated, endothelium-denuded rabbit ductus exposed to neonatal oxygen tension, the maximal effect of a NO donor (SNP) was only 4% of maximal relaxation compared with 80% for PGE$_2$ and 87% for cicaprost (Smith and McGrath, 1993). Consistent with this, L^G-nitro-L-arginine, an inhibitor of eNOS and neuronal NOS (Moncada et al, 1997), at a dosage sufficient to increase mean arterial pressure, had a much smaller contractile effect than indomethacin in the fetal lamb ductus in vivo (Fox et al., 1996). These observations suggest that PGE$_2$ is the most important ductal dilator and that NO may only play an accessory role (Smith and McGrath, 1993; Fox et al., 1996). Given the profound contractile effect of indomethacin on the fetal lamb ductus in vivo (Friedman et al., 1983; Fox et al., 1996), NO

is insufficient on its own to oppose the intrinsic tone of the fetal vessel in utero.

A study in the isolated lamb ductus demonstrated that NO donors had a greater effect than E series PGs in vitro (Walsh and Mentzer, 1987). These experiments were conducted in the absence of indomethacin. Endogenous PGE$_2$ potentiated the sensitivity and maximum response of the rabbit ductus to nonprostanoid vasodilators and inhibited its sensitivity to exogenous PGE$_2$ (Smith and McGrath, 1994), and the absence of indomethacin (and, therefore, the effects of locally released PGE$_2$) probably explains greater sensitivity to NO donors compared with PGE$_2$ (see section X.). The effects of endogenous and exogenous NO on ductal patency have not been studied in the instrumented neonate. This area warrants investigation.

C. Carbon Monoxide

The effects of carbon monoxide (CO) on the ductus have been studied for over a decade in investigations of the mechanism of the oxygen-induced contraction of the ductus (see section IV.). More recently, it has been appreciated that smooth muscle contains an enzyme, heme oxygenase, that can produce CO from heme and that the CO produced may cause vasodilatation through stimulation of cGMP (Morita et al., 1995; Werkstrom et al., 1997) or by effects on potassium channels (Farrugia et al., 1993; Wang and Wu, 1997; Werkstrom et al., 1997).

The expression of the inducible (heme oxygenase-1) and constitutive (heme oxygenase-2) isoforms of the enzyme have been studied by immunohistochemistry in the lamb ductus (Coceani et al., 1997). Heme oxygenase-1 was expressed in both endothelial and smooth muscle cells, whereas heme oxygenase-2 was only found in smooth muscle. The formation of CO from exogenous hemin was blocked by the heme oxygenase inhibitor zinc protoporphyrin IX (ZnPP) (10 μM). However, ZnPP only contracted the ductus exposed to fetal oxygen tension when heme oxygenase-1 had been induced by endotoxin (Coceani et al., 1997). It remains to be established whether CO acts as a dilator of the ductus under physiological conditions in utero.

D. Other Relaxants of the Ductus Arteriosus

In the chronically instrumented fetal lamb, adenosine reversed the contraction of the ductus induced by ventilation of the fetal lungs with oxygen (Mentzer et al., 1985). Furthermore, circulating concentrations of endogenous adenosine varied inversely with both fetal arterial oxygen tension and the degree of contraction of the ductus arteriosus (Mentzer et al., 1985). However, adenosine had no effect on the indomethacin-induced contraction of the fetal lamb in vivo (Friedman et al., 1983) and its maximal effect on the indomethacin-treated, endothelium-denuded rabbit ductus exposed to neonatal oxygen tension was 4% of maximal relaxation compared with 80% for PGE$_2$ (Smith and McGrath, 1993). It is

likely that adenosine has only a minor and accessory role in maintaining patency of the ductus in utero.

The ductus also has β-adrenoceptors that mediate relaxation (Bodach et al., 1980). The contractile effect of catecholamines through α-adrenoceptor activation is offset by its dilator effect through β-adrenoceptors. However, infusion of the β-adrenoceptor antagonist, propranolol, had no effect on ductal patency in the in vivo lamb (Friedman et al., 1983).

IV. Factors Mediating Contraction at Birth

Although the maintenance of patency in utero is an active state and the loss of the dilator effect of PGE_2 is central to the control of the ductus in the neonate (Coceani and Olley, 1988), the trigger to close the vessel after birth is more than just the withdrawal of dilator influences. The major factor actively stimulating contraction is probably the effect of increasing oxygen tension although the isolated ductus is sensitive to a wide range of contractile agonists (see Sections IV.D. and IV.G.). The multiplicity of these contractile systems seems at odds with the relatively simple physiological role of the ductus. This can be explained by the fact that the two main systems that vary at birth, namely oxygen tension and PGE_2, act synergistically to modulate the response of the ductus to vasoconstrictors (Smith and McGrath, 1991, 1993). In the presence of fetal oxygen tension and physiological concentrations of PGE_2, the ductus is virtually unresponsive to even high micromolar concentrations of norepinephrine (Smith and McGrath, 1991). Loss of this profound synergistic inhibition after delivery will uncover the vessel's response to a range of vasoconstrictors.

A. Oxygen-Induced Contraction

In fetal life, the ductus is exposed to an oxygen tension that has been estimated as between 18 to 28 mmHg (Heymann and Rudolph, 1975). After birth, the ductus is exposed to arterial blood because of the reversal of the direction of flow (Dawes et al., 1955) and arterial oxygen tension rises rapidly after delivery (Heymann and Rudolph, 1975). Rising oxygen tension profoundly contracts the ductus (Kennedy and Clark, 1942; Kovalcik, 1963; Fay, 1971). With the exception of the pulmonary circulation, most vascular smooth muscles relax in a low oxygen environment and contract in response to increasing oxygen tension. The response of the ductus to oxygen, although qualitatively similar to other blood vessels, is much greater in magnitude (Heymann and Rudolph, 1975; Smith and McGrath, 1988). Several different mechanisms have been proposed to explain the profound contractile effect of physiological increases in oxygen tension on the ductus. None of the models fit all of the experimental observations, and it is likely that there is more than one oxygen sensor in the ductus. Furthermore, oxygen tension has a profound modulatory effect on other vasoactive systems (Smith and

McGrath, 1991, 1993). It follows, therefore, that the effects of locally released vasoactive agents that exert a tonic effect on ductal contractility will vary with alterations in oxygen tension.

1. Cytochrome a_3 hypothesis. Fay (1971) demonstrated some fundamental properties of the oxygen-induced contraction of the guinea pig ductus: exposure of the vessel to increasing oxygen tension across the range 0 to 140 mmHg induced incremental contraction; the effect was the same when either the luminal or the adventitial side of the ductus was exposed to elevated oxygen; and local anesthetics and tetrodotoxin (inhibitors of nerve-mediated effects) had no effect on the response. These findings suggested that oxygen acts directly on the smooth muscle cells of the ductus, which was consistent with the lack of effect of a range of antagonists of autonomic nervous system mediators on the oxygen-induced contraction of the lamb and guinea pig ductus (Kovalcik, 1963). In addition, Fay observed that the oxygen-induced contraction was inhibited by cyanide and several other inhibitors of oxidative phosphorylation (Fay, 1971; Fay and Jobsis, 1972). Cyanide had little effect on the acetylcholine-induced contraction, although some of the other inhibitors of oxidative phosphorylation reduced the response to acetylcholine. Fay postulated that cytochrome a_3 was the oxygen sensor and that the contractile effect of oxygen was related to adenosine triphosphate (ATP) levels in ductal smooth muscle cells (Fay and Jobsis, 1972). Subsequently, it was demonstrated that CO had a greater affinity than oxygen for the target hemoprotein in the ductus (Coceani et al., 1984). Because oxygen has a nine-fold greater affinity for cytochrome a_3 than CO (Ball et al., 1951), it was concluded that CO was acting at a different hemoprotein (Coceani et al., 1984).

Cytochrome a_3 is only one of many hemoproteins with a role in smooth muscle contractility. Consequently, cyanide affects vascular smooth muscle contraction by several membrane-dependent and membrane-independent mechanisms, e.g. activating ATP-sensitive potassium channels (Lydrup et al., 1994; Beech et al., 1993), activating large conductance calcium-sensitive potassium channels (Millerd et al., 1993), and by inhibiting iNOS (Hobbs et al., 1994). Although the apparently selective effect of cyanide on the oxygen-induced contraction is interesting, it sheds little light on the potential mechanism by which oxygen exerts its contractile effect.

2. Arachidonate hypothesis. Given that dilation of the ductus in utero is mediated by an AA metabolite (PGE_2), it was an attractive hypothesis that an AA metabolite might also mediate oxygen-induced contraction of the vessel with a shift in AA metabolism occurring at birth. Furthermore, the oxygen-induced contraction of the human umbilical artery was blocked by PGHS inhibitors (McGrath et al., 1986) and was found to be mediated by TxA_2 (Templeton et al., 1991). However, the oxygen-induced contraction of the lamb ductus arteriosus was

not blocked by PGHS (Coceani et al., 1979) or lipoxygenase (Coceani et al., 1982) inhibitors. The remaining major enzymatic AA pathway is the formation of epoxides by cytochrome P_{450} (also called the monooxygenase or epoxygenase pathway), which generates contractile agonists in other vascular smooth muscles (see Harder et al., 1995 for review). Although there is some evidence to suggest that inhibiting cytochrome P_{450} relaxes the ductus (see Section IV.A.3.), there are several pieces of evidence to suggest that this is not mediated by preventing synthesis of a constrictor epoxide. First, in the presence of combined PGHS and lipoxygenase inhibition, exogenous AA only relaxed the ductus in elevated oxygen tension (Coceani et al., 1988). Second, a range of AA epoxides were without effect or caused relaxation of the isolated lamb ductus (Coceani et al., 1988). And third, no monooxygenase activity could be demonstrated in the closing lamb ductus (Coceani et al., 1994a).

There is a nonenzymatic AA pathway, namely, free radical catalyzed peroxidation forming isoprostanes, such as 8-epi-$PGF_{2\alpha}$. Isoprostanes have been shown to contract vascular smooth muscle. Initially they were thought to act through the TP receptor, but there is recent evidence for a novel receptor (see Roberts and Morrow, 1997 for review). However, this pathway is unlikely to mediate the oxygen-induced contraction of the ductus because free radicals have a dilator effect on the vessel in the absence of indomethacin and neither free radicals nor scavengers affect ductal tone in the presence of indomethacin and elevated oxygen tension (Clyman et al., 1989b).

3. Endothelin/cytochrome P_{450} hypothesis. Inhibition of the oxygen-induced contraction of the ductus by cyanide (Fay, 1971) implied the role of a hemoprotein. It was found that CO relaxed the oxygen contracted ductus and this effect was reversed by monochromatic light at a peak of 450 nM (Coceani et al., 1988). It was concluded that cytochrome P_{450} was the oxygen sensor and its activation promoted contraction. As discussed above (see Section IV.A.2.), if the enzyme has a role, it is unlikely to be acting through formation of a constrictor AA epoxide (Coceani et al., 1988, 1994a). The effect of oxygen on the enzyme is proposed to lead to the release from endothelial and smooth muscle cells of endothelin (ET)-1, which causes contraction of the ductus mediated by the ET_A receptor (Coceani et al., 1992). There is no currently known nonepoxide mechanism linking cytochrome P_{450} to the production of ET-1, although conformational change in the enzyme or the production of a nonepoxide stimulatory metabolite have been proposed (Coceani, 1994).

There is a large body of evidence obtained from studies on the lamb ductus arteriosus, mostly from a single laboratory, which supports the cytochrome P_{450}/ET-1 hypothesis (Coceani, 1994). Immunohistochemistry has identified a glucocorticoid-inducible cytochrome P_{450} enzyme that is present in the fetal ductus but not aorta (Coceani et al., 1994a). CO (an inhibitor of cytochrome P_{450} with activity at other hemoproteins) and 1-aminobenztriazole (ABT) (an inhibitory suicide substrate for cytochrome P_{450}) relaxed the oxygen contracted ductus (Coceani et al., 1988, 1996b). The effect of CO was reversed by monochromatic light with a maximum effect at 450 nM (Coceani et al., 1988). Both CO and ABT had less of an effect on the potassium contracted ductus exposed to fetal oxygen tension than the oxygen-contracted ductus (Coceani et al., 1984, 1996b). This series of findings supports the concept that the oxygen-induced contraction of the ductus is dependent on cytochrome P_{450}.

The endothelium and smooth muscle of the ductus synthesized ET-1 (Coceani and Kelsey, 1991) and ET-1 was found to be a potent constrictor of the lamb ductus (Coceani et al., 1989b). CO inhibited the release of ET-1 by the endothelium-denuded ductus to undetectable levels (Coceani and Kelsey, 1991). The ET_A receptor antagonist BQ-123 and the ET converting enzyme inhibitor, phosphoramidon, both reduced the oxygen-induced contraction of the ductus (Coceani et al., 1992). This series of findings supports the concept that ET-1 mediates the contractile effect of increasing oxygen tension and that ET-1 release is controlled by cytochrome P_{450}.

However, the series of experiments that generated the findings above also produced several observations that complicate the proposed model. First, the dilator effects of CO and ABT on the indomethacin-induced contraction in 0% and 2.5% (respectively) oxygen were of a similar magnitude to their effects on the oxygen-induced contraction (Coceani et al., 1984, 1996). Second, metyrapone, another chemical inhibitor of cytochrome P_{450}, actually had a greater dilator effect on the ductus exposed to fetal compared with neonatal oxygen tension (Coceani et al., 1984). These findings suggest that the dilator effect of putatively selective cytochrome P_{450} inhibitors may well be unrelated to the mechanism of the oxygen-induced contraction.

The contractile response to oxygen persisted in the presence of four other structurally unrelated cytochrome P_{450} inhibitors, and with 3 of them the magnitude of the contraction was unchanged (Coceani et al., 1988). The inconsistent findings with other cytochrome P_{450} inhibitors suggest that the effect of CO may not be mediated by inhibition of cytochrome P_{450}. CO has been shown to relax other smooth muscles by several mechanisms, including stimulating guanylate cyclase (Utz and Ullrich, 1991; Morita et al., 1995), and activating both voltage sensitive (Werkstrom et al., 1997) and Ca–activated (Farrugia et al., 1993) potassium channels by cGMP-independent mechanisms.

In the lamb ductus, it was found that CO approximately doubled the levels of cGMP in the intact and endothelium-denuded lamb ductus in the absence of a NOS inhibitor and, in the presence of a NOS inhibitor, levels of cGMP rose five-fold with exposure to CO (Coce-

ani et al., 1996a). Furthermore, the effect of CO on cGMP could be fully reversed by monochromatic light with a wavelength of 450 nM (Coceani et al., 1996a). However, the absolute magnitude of the changes in cGMP were small when related to the magnitude of relaxation and when compared with the changes in cGMP induced by SNP causing a similar degree of relaxation (Coceani et al., 1996a).

In 0% oxygen, CO only caused 38% reversal of the potassium contracted ductus (Coceani et al., 1984). Given that CO completely reversed the indomethacin and oxygen-induced contractions, the dilator effect of CO may be specifically inhibited by elevated potassium, which would be consistent with CO activating potassium channels, as demonstrated in other smooth muscles (Farrugia et al., 1993; Morita et al., 1995). The effects of CO on potassium currents in the ductus have not been studied.

The observations above raise the possibility that the effects of CO are not mediated through interaction with cytochrome P_{450}. The parallels in the effects of CO and ABT are more supportive of a P_{450}-mediated effect (Coceani et al., 1996b). However, ABT has also been shown to relax rat aortic and pulmonary vascular smooth muscle by a cytochrome P_{450}-independent mechanism (Chang et al., 1992).

There is other evidence that questions the role of ET-1 in mediating the contractile effect of oxygen. Infusion of ET-1 into the fetal lamb did not contract the ductus arteriosus (Chatfield et al., 1991), although ventilation of the fetal lungs with oxygen did induce ductal contraction (Mentzer et al., 1985). Increasing oxygen from 2.5% to 95% had no statistically significant effect on ET-1 release from the intact isolated ductus (Coceani et al., 1992). In vitro, high concentrations of exogenous ET-1 did not fully reverse CO-mediated relaxation of the isolated ductus (Coceani et al., 1989a). Although CO inhibited ET-1 release from the endothelium-denuded ductus, it had only a transient effect on ET-1 release from the intact vessel (Coceani and Kelsey, 1991). Furthermore, although 40 μM ABT reduced tone by over 50% in the ductus exposed to neonatal (and fetal) oxygen tension, 1000 μM ABT had no statistically significant effect on ET-1 release from either the endothelium-denuded or intact ductus (Coceani et al., 1996b). Finally, the ET_A antagonist BQ-123 caused a similar proportional reduction in tone of the ductus exposed to fetal oxygen tension compared with the vessel exposed to neonatal oxygen tension (Coceani et al., 1992).

From the foregoing, it is not yet possible to define fully the relative roles of cytochrome P_{450} and ET-1 in mediating the oxygen-induced contraction of the ductus. It seems likely that both a cytochrome P_{450} enzyme and local release of ET-1 have a tonic effect on the contractility of the ductus arteriosus. The absolute amount of tone induced by these systems is likely potentiated in elevated oxygen tension as oxygen tension potentiates

the response of the ductus to vasoconstrictors (Ikeda et al., 1973a; Smith and McGrath, 1988, 1991). It may be that the oxygen sensor primarily exists elsewhere in ductal smooth muscle cells and the activity of a distinct oxygen sensitive system modulates the tonic effect of cytochrome P_{450} and/or ET-1. Furthermore, the minimal effect of oxygen, CO, and ABT on ET-1 release from the intact ductus undermine the proposal that oxygen and/or cytochrome P_{450} activity controls ET-1 release. Given the now well-characterized role of voltage sensitive potassium channels as a ductal oxygen sensor (see Section IV.A.4.), the effect of cytochrome P_{450} inhibitors and ET-1 on potassium channels warrants study, especially because cytochrome P_{450} control of voltage sensitive potassium channels appears to function as a mediator of the effects of oxygen tension in pulmonary vascular smooth muscle (Yuan et al., 1995).

4. Membrane hypothesis. It was found that the oxygen-induced contraction of the guinea pig ductus arteriosus was associated with smooth muscle cell depolarization (Roulet and Coburn, 1981). Subsequently, it was observed that glibenclamide, a blocker of ATP-sensitive potassium channels, contracted the isolated rabbit ductus exposed to fetal oxygen tension and had little effect on the oxygen-contracted ductus (Nakanishi et al., 1993). Furthermore, cromakalim, an ATP-sensitive potassium channel activator, relaxed the ductus contracted by oxygen, but had much less of an effect on the vessel precontracted with 10 μM norepinephrine (Nakanishi et al., 1993). These authors proposed that oxygen depolarized the ductus by closing ATP-sensitive potassium channels.

Further studies on smooth muscle cells from the rabbit ductus using patch clamp techniques confirmed that increasing oxygen tension inhibited whole cell potassium current (Tristani-Firouzi et al., 1996). However, when the effects of antagonists of potassium channels were studied using isolated ring preparations, it was found that 1 mM 4-aminopyridine (AP) (an inhibitor of delayed rectifier potassium channels) caused a much greater contraction of the rabbit ductus exposed to fetal oxygen tension than 10 μM glibenclamide (Tristani-Firouzi et al., 1996). Furthermore, whereas 4-AP (in ductal smooth muscle cells exposed to fetal oxygen tension) suppressed whole cell potassium current and depolarized ductal smooth muscle cells, glibenclamide did not. Finally, single channel recordings demonstrated a 58-pS channel that was closed both by 4-AP and increasing oxygen tension (Tristani-Firouzi et al., 1996). These observations suggested that increasing oxygen contracts the ductus at least in part by closing oxygen-sensitive delayed rectifier potassium channels.

Increased oxygen tension and subsequent depolarization was associated with a rise in intracellular calcium in the rabbit ductus (Nakanishi et al., 1993; Tristani-Firouzi et al., 1996); this is likely because of an influx of calcium from the extracellular fluid as the oxygen-in-

duced contraction of the rabbit ductus was dependent on the presence of extracellular calcium, was inhibited by calcium channel antagonists to a similar extent as the potassium-induced contraction, and was largely unaffected by the antagonist of intracellular calcium release, ryanodine (Nakanishi et al., 1993). The oxygen-induced contraction of the isolated rabbit ductus was inhibited by specific antagonists of L-type calcium channels such as verapamil, diltiazem, and nisoldipine, whereas a T-type calcium channel inhibitor, mibefradil (Ro40–5967), had no effect (Nakanishi et al., 1993; Tristani-Firouzi et al., 1996). Although nickel inhibited the oxygen-induced contraction, it had a minimal effect at 500 μM and only caused significant inhibition in the millimolar range (Nakanishi et al., 1993). Given that its IC_{50} for blocking T-type channels is about 6 μM (Mlinar and Enyeart, 1993), it is reasonable to conclude that entry of calcium from the extracellular space is through L-type calcium channels. Calcium influx appears to have a role in mediating closure of the ductus as verapamil delayed closure of the ductus in the neonatal rat (Takizawa et al., 1994b).

The potassium channel model is only part of the explanation for the contractile effect of oxygen on the ductus because oxygen can still cause contraction of the guinea pig vessel in the presence of 126 mM extracellular potassium (Roulet and Coburn, 1981), i.e., oxygen also has a membrane-independent contractile effect on the ductus.

5. Characterizing an oxygen sensor. I propose that the definition of "sensor" in this context is a system that is directly altered (e.g. synthesis or release of a vasoactive agent from the ductus, or the state of an ion channel) by increasing oxygen tension in such a way as to promote contraction. Oxygen profoundly influences the response of the ductus to a wide range of vasodilators (Smith et al., 1993) and vasoconstrictors (Ikeda et al., 1973a; Smith and McGrath 1988, 1991). Indeed, these studies failed to identify a vasoactive agent whose effect on the ductus was not affected by oxygen tension. It follows, therefore, that when an agent has a tonic effect on the ductus (dilator or contractile), the magnitude of its effect is likely to vary with oxygen tension, i.e., the change is secondary to altered sensitivity to the effect of the given system rather than an activation (or inhibition) of the system per se. Therefore, an agonist with a tonic effect on ductal tone may contribute to the oxygen-induced contraction but not be an oxygen sensor.

For instance, the dilator effect of ABT on the oxygen-induced contraction of the lamb ductus is central to the proposal that oxygen contracts the ductus by stimulating cytochrome P_{450} (Coceani et al., 1996b). However, in the presence of a constant state of cytochrome P_{450} activation, if the effect of cytochrome P_{450} is potentiated in elevated oxygen tension, as might be expected by studies with contractile agonists (Ikeda et al., 1973a; Smith and McGrath, 1988, 1991), administration of ABT would be expected to cause a greater relaxation in elevated oxygen tension compared with fetal oxygen tension, even if oxygen had no effect on cytochrome P_{450} activity. Therefore, an increased magnitude of effect of an antagonist of a system in the presence of elevated oxygen tension is not, on its own, sufficient to establish that system as an oxygen sensor, as defined above.

There is an interesting parallel between the effects of fetal and neonatal oxygen tension on the ductus and the effects of PGE_2 and PGHS inhibitors. PGE_2, like fetal oxygen tension, increases the sensitivity of the ductus to vasodilators (Smith and McGrath, 1994) and decreases its sensitivity to a range of vasoconstrictors (Smith and McGrath, 1991, 1994). Conversely, indomethacin, like increasing oxygen tension, increases the sensitivity of the ductus to vasoconstrictors (Smith and McGrath, 1988) and decreases its sensitivity to vasodilators (Smith and McGrath, 1994). From the foregoing, PGE_2, like oxygen tension, would appear to act at a fundamental cellular level in the ductus, and this has been confirmed in α-toxin permeabilized ductal smooth muscle from fetal rabbits (Crichton et al., 1997). This predicts that antagonists of systems that exert a tonic effect on ductal contractility might have a similar effect on the indomethacin-induced contraction of the ductus exposed to fetal oxygen tension as on the oxygen-induced contraction of the vessel. As discussed above (see Section IV.A.3.), it has been observed that both CO and ABT are as effective at inhibiting the indomethacin-induced contraction of the vessel exposed to fetal oxygen tension as the oxygen-induced contraction of the lamb ductus (Coceani et al., 1984, 1996b).

B. Contractile Effects of prostaglandins

Two early in vitro studies failed to demonstrate significant contractile effects of prostanoids on the ductus. The first looked at the effect of a single high concentration (about 7 μM) of $PGF_{2\alpha}$ that was found to cause a small contraction of the bovine ductus exposed to either fetal or neonatal oxygen tension (Starling and Eliott, 1974). The other study (Coceani et al., 1978a) incubated PGG_2 or PGH_2 with microsomal fractions of human platelets or guinea pig lungs to generate TxA_2. PGG_2 or PGH_2 on their own relaxed the ductus (presumably by the formation of PGE_2), but when PGG_2 or PGH_2 were incubated in platelet or lung microsomes, they were without effect.

More recently, the effect of stable synthetic agonists of contractile prostanoid receptors have been studied (Smith and McGrath, 1995). It was demonstrated that the fetal rabbit ductus arteriosus has two prostanoid receptors coupled to contractile pathways: namely, a TP receptor and a contractile EP receptor, probably EP_3. The TP receptor agonist, U46619 (5-heptenoic acid, 7-[6-(3-hydroxy-1-octenyl)-2-oxabicyclo[2.2.1]hept-5-yl-,[1R-[1α,4α,5β,(Z),6α,(1E, 3S*)]]-), contracted the ductus in the nanomolar range, had a maximal effect similar to

norepinephrine, and was antagonized by a TP receptor antagonist. The ductus also contracted in response to nanomolar concentrations of the selective EP_3 agonists GR63799X ([1R-[1a(Z),2b(R*), 3a]]-4-(benzoylamino) phenyl 7-[3-hydroxy- 2 (2-hydroxy- 3- phenoxypropoxy)- 5- oxocyclopentyl]- 4- heptenoate) and sulprostone. The maximum response to these agonists was smaller than the response to U46619. Interestingly, 10 nM sulprostone decreased the sensitivity of the ductus to the dilator effect of PGE_2, whereas 10 nM U46619 did not. Therefore, the ductus arteriosus has both dilator and contractile receptors to PGE_2, and the contractile receptor modulates the effects of the dilator receptor (Smith and McGrath, 1995). The significance of this observation is discussed in section IV.C.2.

C. Elimination of Dilator Prostaglandins

Loss of the dilator effect of PGE_2 is central to the closure of the ductus, and treatment of the neonatal rat with PGE_2 is sufficient on its own to prevent postnatal closure (Jarkovska et al., 1992). The relative roles of withdrawal of locally released and circulating PGE_2 remain to be determined.

1. Circulating prostaglandin E_2. The high fetal circulating concentrations of PGE_2 fall dramatically after birth, by ten-fold at 1 h and by twenty-fold at 3 h, in the term lamb (Clyman et al., 1980b). Loss of the dilator effect of circulating PGE_2 has been postulated to be fundamental to the closure of the ductus (Clyman, 1987). The fall in circulating concentrations of PGE_2 is because of (*a*) the increase in lung blood flow that occurs at birth because the lungs are the major site of PG catabolism (Tsai and Brown, 1987) and (*b*) the loss of the placenta, the major source of circulating PGE_2 in the fetus (see Thorburn, 1992).

2. Locally released prostaglandin. The elimination of the dilator effects of locally released PGs after birth is more complex and less well understood than for circulating PGE_2 (Clyman, 1987). Paradoxically, increasing oxygen tension stimulates PGE_2 release by the lamb ductus (Clyman et al., 1980a; Coceani et al., 1986) i.e., oxygen tension, which contracts the vessel, stimulates the release of PGE_2, which relaxes it. This apparent anomaly has been resolved at least in part with the appreciation of the contractile effects of PGs on the ductus (section IV.)

Increasing oxygen tension inhibited PGI_2-synthase in the lamb ductus to a much greater extent than in the fetal lamb aorta (Needleman et al., 1981). Inhibition of this enzyme would result in decreased formation of PGI_2, which would be expected to promote contraction of the ductus (Smith and McGrath, 1993, 1994; Smith et al., 1994). Inhibition of PGI_2-synthase would also be expected to lead to accumulation of PGH_2, which may stimulate ductal contraction directly because PGH_2 is one of several endogenous agonists of the TP receptor (Coleman et al., 1994b). Increased release of PGE_2 in the presence of elevated oxygen tension may be secondary to accumulation of its precursor, PGH_2.

Release of PGE_2 itself may also promote contraction through the contractile EP_3 receptor (Smith and McGrath, 1995). Increasing oxygen tension decreases the sensitivity of the ductus to the dilator action of PGE_2 and potentiates its response to vasoconstrictors (Smith and McGrath, 1991, 1993). The balance between the dilator effect of PGE_2 through the EP_4 receptor and its contractile effect through the EP_3 receptor is likely to change after birth with increasing activation of the contractile pathway.

D. Neural Vasoconstriction

The ductus arteriosus of all species studied is innervated by catecholamine containing nerves (Boreus et al., 1969; Ikeda, 1970; Ikeda et al., 1972; Bodach et al., 1980). The catecholamine content of the lamb ductus was similar to peripheral arteries that are known to be under autonomic neural control (Ikeda et al., 1972). The guinea pig and lamb ductus contracted in response to transmural stimulation of nerves (Ikeda et al., 1973a; Bodach et al., 1980), and the ductus of several species contracted in response to exogenous norepinephrine (Kovalcik, 1963; Aronson et al., 1970; Smith and McGrath, 1988). The effect of transmural stimulation on the guinea pig ductus was potentiated by raised oxygen tension, as was the response of the vessel to exogenous norepinephrine (Ikeda et al., 1973a). The effect of transmural stimulation was blocked in part by α-adrenoceptor blockade (Bodach et al., 1980), and the treatment of the pregnant guinea pig with phenoxybenzamine (a nonselective α-adrenoceptor antagonist) delayed closure of the ductus in the offspring (Hornblad and Larsson, 1972). The α-adrenoceptor subtype mediating the contractile effect of norepinephrine on the ductus has not been elucidated.

The guinea pig and human ductus also contracted in response to acetylcholine (Kovalcik, 1963; McMurphy and Boreus, 1971; Ikeda et al., 1973a), and the lamb ductus is innervated with acetylcholine-containing nerves (Silva and Ikeda, 1971). Atropine blocked the contractile response of the ductus to exogenous acetylcholine, but had no effect on the contraction induced by transmural stimulation of nerves of the isolated lamb ductus arteriosus (Bodach et al., 1980).

The central control of activity of these pressor nerves is unknown. Interestingly, the ductus of several species has structures in its wall that are similar to the carotid and aortic bodies (Boyd, 1941; Fay, 1971; MacDonald et al., 1983) and send afferent fibers to the left vagus nerve (Boyd, 1941). There is no information on what physiological stimuli might activate this apparent sensory system, but the presence of afferent and efferent neural pathways in the ductus suggests the possibility of a control loop.

E. Other Locally Released Vasoconstrictors

The potential role of ET-1 is discussed in section IV.A.3. The adventitia of the guinea pig ductus has many mast cells present, which can release other vasoconstrictors such as histamine and 5-hydroxytryptamine (Fay, 1971), both of which contracted the isolated ductus of several species (Aronson et al., 1970; McMurphy and Boreus, 1971; Smith and McGrath, 1991). The factors that control degranulation of ductal mast cells are not known. Infusion of 5-hydroxytryptamine into the chronically instrumented fetal lamb had no effect on ductal patency (Friedman et al., 1983), and this may reflect the profound synergistic dilator effect of fetal oxygen tension and PGE_2 on the response of the ductus to vasoconstrictors (Smith and McGrath, 1991).

F. Myogenic Tone

Isolated rings of guinea pig ductus arteriosus contracted in response to stretch (Ikeda et al., 1973a), and isolated perfused rabbit ductus contracted in response to increased perfusion pressure (Kriska et al., 1990). Myogenic tone of both isolated rings of rabbit ductus and the isolated perfused vessel was inhibited by both endogenous and exogenous PGs (Kriska et al., 1990; Smith, 1997). However, the role of myogenic tone in the physiological control of the ductus is as yet obscure.

G. Circulating Vasoconstrictors

Enalapril, an angiotensin converting enzyme inhibitor, delayed ductal closure when given to fetal rats and could reopen the closed ductus when given at 3 h of life (Takizawa et al., 1994a,c). Surprisingly, the effects of angiotensin II on the isolated ductus have not been studied, but the drug had no effect on the patency of the vessel when infused into the chronically instrumented fetal sheep (Friedman et al., 1983). There have been several reports linking maternal consumption of angiotensin converting enzyme inhibitors with patent ductus arteriosus (PDA) in the neonate, but a systematic review of the literature did not support an association, although the drugs in clinical use can cross the placenta (Hanssens et al., 1991).

The ductus contracts in response to other circulating vasoactive agents, such as epinephrine (Kovalcik, 1963), through α-adrenoceptors, and bradykinin (Kovalcik, 1963; Aronson et al., 1970). The latter was released after ventilation of the lungs with oxygen in the lamb (Heymann et al., 1969), and the levels of bradykinin in human cord blood were higher than in the adult (Melmon et al., 1968). The rat ductus also contracted in response to steroid hormones, namely, corticosteroids (Momma et al., 1981) and progesterone (Pulkkinen et al., 1986). The contractile effect of corticosteroids is probably related to modulating the sensitivity of the ductus to PGE_2 (see section V.B.). The mechanism of action of progesterone is unknown, but, unlike corticosteroids (Momma and

Takao, 1989a), it does not interact with the effect of indomethacin (Pulkkinen et al., 1986).

V. Ontogeny of Pharmacological Responses

As might be expected, the ductus undergoes alterations in its pharmacological responsiveness with advancing gestational age. The procontractile pathways are clearly held in check at term, however, because the ductus is widely patent immediately before and after delivery (e.g. Momma et al., 1980). It may be that the increasing concentrations of PGE_2 in fetal blood with advancing gestational age (Clyman et al., 1980b) provide a parallel increase in inhibition that is then lost at birth by the mechanisms discussed in section IV.C.1.

A. Altering Pharmacological Responses with Advancing Gestational Age

The contractile effect of indomethacin on the ductus varies with gestational age, but the findings are different when in vitro and in vivo studies are compared. The contractile effect of indomethacin and ibuprofen on the isolated lamb ductus decreased towards term (Clyman et al., 1978a; Coceani et al., 1979), whereas the contractile effect of indomethacin in vivo increased with advancing gestational age in fetal rats, lambs, and humans (Friedman et al., 1983; Momma and Takao, 1987; Moise et al., 1988). The discrepancy between the findings in vivo and in vitro may be because of the additional dilator effect of circulating PGE_2 in vivo. The sensitivity of the lamb ductus to PGE_2 and PGI_2 decreased towards term (Clyman et al., 1980c). The preterm lamb ductus had greater PGI_2 synthase activity than term (Clyman et al., 1978b), but released less PGE_2 than the term ductus (Clyman et al., 1979). The increased activity of PGI_2 synthase in the preterm ductus may result in less accumulation of PGH_2, and, because PGE_2 is formed from PGH_2, it may explain the low levels of PGE_2 released. The main catabolic pathway for circulating PGE_2 is 15(OH)PGDH: the activity of this enzyme in the fetal rat and rabbit lung increased towards term (Simberg, 1983; Tsai and Einzig, 1989).

There is also maturation of non-PG procontractile pathways with advancing gestational age. The contractile effect of transmural stimulation of pressor nerves in the isolated guinea pig ductus increased towards term (Ikeda et al., 1973b), as did the oxygen-induced contraction of the guinea pig and lamb vessels (Noel and Cassin, 1976; Clyman et al., 1979). The reduced contractile response of the preterm lamb ductus to oxygen may reflect greater inhibition by locally released PGs because the combined contractile effect of oxygen and indomethacin did not differ significantly comparing the preterm and term vessels (Clyman et al., 1980c). However, a recent preliminary report of patch clamp studies of smooth muscle cells from the rabbit ductus found that the potassium channels controlling membrane potential change from the oxygen-insensitive Ca-activated chan-

nel to the oxygen-sensitive delayed rectifier channel with advancing gestational age (Reeve et al., 1997). This suggests that the fundamental pathway involved in the oxygen-induced contraction changes with advancing gestational age rather than merely a reduced degree of inhibition of the oxygen-induced contraction by locally released PG.

B. The Effects of Corticosteroids on Pharmacological Responses

The sensitivity of the preterm lamb ductus to PGE_2 and PGI_2 is decreased by antenatal administration of corticosteroids to the mother (Clyman et al., 1981b), whereas, antenatal administration of hydrocortisone had no effect on PGE_2 release from the isolated preterm lamb ductus (Clyman et al., 1981a). Activity of pulmonary 15(OH)PGDH can be induced in the preterm animal by the administration of hydrocortisone (Tsai and Brown, 1987). Again, non-PG procontractile pathways appear to be enhanced by corticosteroids because the oxygen-induced contraction of the preterm lamb ductus was increased by antenatal administration of hydrocortisone (Clyman et al., 1981b). The effects of antenatal corticosteroids on the expression of potassium channels in the preterm ductus have not been reported, and this area warrants study.

The molecular and cellular mechanisms of the effects of corticosteroids on the ductus have not been examined. Given that the pharmacological responses of the ductus are reasonably well understood, the vessel may be an ideal model for studying the fundamental mechanisms by which corticosteroids affect the preterm fetus, an area of profound clinical importance.

VI. Intracellular Control of Contractility

A. Control of Membrane Potential and Intracellular Calcium

As discussed above (see Section IV.A.4.), oxygen tension depolarizes the ductus arteriosus, which is likely to be because of closure of delayed rectifier potassium channels. Ductal smooth muscle cells also have ATP-sensitive potassium channels as judged by the contractile effect of glibenclamide on isolated rings of rabbit ductus exposed to fetal oxygen tension (Nakanishi et al., 1993; Tristani-Firouzi et al., 1996) and the dilator effect of cromakalim in the same preparation (Nakanishi et al., 1993; Smith and McGrath, 1993, 1994). The fetal rabbit ductus arteriosus is much more sensitive to cromakalim than adult aorta; the drug causes half maximal relaxation in the ductus precontracted with 10 μM norepinephrine and elevated oxygen tension at a concentration that was subthreshold in the adult rabbit aorta precontracted with 0.1 μM norepinephrine (Bray et al., 1991; Smith and McGrath, 1994).

Calcium channel blockers abolished the oxygen-induced contraction when the vessel was exposed to the drug before increasing oxygen tension (Nakanishi et al., 1993; Tristani-Firouzi et al., 1996). However, nifedipine had no effect on the sustained contraction of the isolated fetal rabbit ductus to indomethacin, elevated oxygen tension, and 10 μM norepinephrine (Smith and McGrath, 1993). This may reflect the presence of indomethacin and/or norepinephrine, or it may be that calcium influx is a feature of the initiation of ductal contraction by oxygen, but not its sustained response, as has been demonstrated for the response of the rabbit aorta to norepinephrine (Bray et al., 1991). It would be interesting to examine the effect of exposing the ductus to calcium channel blockers after sustained exposure to increased oxygen tension.

Other than the lack of effect of ryanodine on the oxygen-induced contraction (Nakanishi et al., 1993), the release of calcium from intracellular stores in the ductus has not been studied.

B. Other Signal Transduction Systems

With the exception of calcium, there are no studies on the role of contractile second-messenger systems in the control of the ductus, such as the inositol triphosphate cascade and protein kinase C. It would be particularly interesting to establish whether varying oxygen tension acts in part by activating these systems.

There is more information regarding the second-messenger systems mediating relaxation of the ductus. As discussed in section III, it is likely that the effects of PGE_2 are mediated through stimulation of AC. The cellular mechanism of cAMP in the ductus have been studied in the staphylococcal α-toxin permeabilized smooth muscle from the fetal rabbit ductus. It was found that both forskolin (a direct activator of AC) and exogenous cAMP decreased the sensitivity of the contractile proteins to calcium (Crichton et al., 1997). In other tissues, cAMP can also affect intracellular concentrations of calcium (Karaki et al., 1997), but this has not been studied in the ductus.

NO donors increased both cAMP and cGMP in the isolated lamb ductus (Walsh and Mentzer, 1987). It is unclear whether the effect on AC was a direct effect of NO or was secondary to a release of dilator PGs because these experiments were not conducted in the presence of an inhibitor of endogenous PG synthesis. The cellular mechanisms of cGMP mediated-relaxation have not been studied in the ductus.

The relative importance of the cAMP and cGMP has been studied in the isolated fetal rabbit ductus arteriosus. Forskolin completely reversed the combined contractile effects of elevated oxygen tension, 10 μM norepinephrine, and 1 μM indomethacin, whereas SNP only caused 4% of the effect of forskolin (Smith and McGrath, 1993); this suggests that AC is more important than guanylate cyclase in maintaining ductal patency in utero.

C. Contractile Proteins

Although the intracellular calcium concentration is clearly critical in mediating the oxygen-induced contraction of the ductus, recent work has suggested that responsiveness of the contractile proteins themselves may also have an important role in mediating contraction at birth. It has been demonstrated that the fetal rabbit ductus permeabilized with staphylococcal α-toxin was more sensitive to calcium than fetal rabbit aorta, pulmonary artery, and a diverse range of vascular and nonvascular smooth muscles from adult animals (Crichton et al., 1997). The precise mechanism of this remains to be resolved, but it might reflect an intrinsically high sensitivity to calcium of ductal contractile proteins or stimulation of ductal calcium sensitivity under the conditions employed.

Considering the first possibility, it has been demonstrated that the ductus arteriosus expresses a "precociously mature" phenotype compared with other fetal vascular smooth muscles in that it expresses an adult specific vascular smooth muscle myosin heavy chain isoform, SM2 (Kim et al., 1993; Sakurai et al., 1996; Colbert et al., 1996); this isoform is not expressed in fetal rabbit aorta and pulmonary artery and only begins to be expressed in these vessels after birth (Sakurai et al., 1996). The expression of the SM2 isoform in the fetal rabbit ductus arteriosus is temporally and spatially associated with signaling by endogenous retinoic acid (Colbert et al., 1996). There is no information on the relationship between the ratio of the SM1/SM2 isoforms and calcium sensitivity in vascular smooth muscle. However, it is unlikely that the high level of SM2 expression completely explains the increased ductal sensitivity to calcium because the ductus was more sensitive to calcium than adult vascular smooth muscles (Crichton et al., 1997), which also express the "mature" phenotype. The umbilical arteries also contract profoundly at birth by an oxygen-induced mechanism (McGrath et al., 1986). Interestingly, these are the only other sites of SM2 expression in fetal vascular smooth muscle (Kim et al., 1993); this supports the interpretation that expression of the SM2 isoform in the ductus may have a role in its postnatal closure.

The ductus from the second trimester human fetus was also found to have "advanced differentiation" of smooth muscle cells compared with the aorta in terms of the degree of expression of the actin binding proteins calponin and caldesmon. The neonatal aorta and ductus had similarly high levels of expression of these proteins, but the fetal ductus had greater levels of expression than the fetal aorta (Slomp et al., 1997). However, because these proteins tend to decrease the calcium sensitivity of permeabilized smooth muscle preparations (see Karaki et al., 1997), the relationship with the enhanced calcium sensitivity of the ductus remains obscure.

Increased sensitivity of ductal smooth muscle to calcium may indicate that it was activated by some mechanism under the conditions studied. It is possibly significant that these experiments were conducted at ambient oxygen tension (Crichton et al., 1997), i.e., physiologically elevated for the ductus. If increased calcium sensitivity was secondary to exposure to elevated oxygen tension, it would necessarily be an effect of oxygen that was independent of membrane potential and, as discussed above (see Section IV.A.4.), there is evidence to support the presence of an oxygen sensor that acts independently of changes in membrane potential (Roulet and Coburn, 1981), and this would also predict that the technique used to skin the ductal smooth muscle might alter its calcium sensitivity. Saponin is a detergent that disrupts the integrity of the cell membranes to a much greater extent than α-toxin; the latter preserves its response to receptor-mediated events, whereas these are abolished by saponin. Smooth muscle from the lamb ductus skinned with saponin was less sensitive to calcium than smooth muscle from the rabbit ductus skinned with α-toxin, and varying oxygen tension had no effect on the calcium sensitivity of the saponin skinned smooth muscle (Coceani et al., 1989b). It is plausible that oxygen might increase the sensitivity of ductal smooth muscle to calcium by a cell-membrane coupled mechanism. This hypothesis could be tested by establishing the effect of oxygen tension on the calcium sensitivity of the α-toxin permeabilized ductus.

VII. Ductal Remodeling

A. Anatomical Changes After Birth

Given that the ductus changes from an artery conveying 60% of the combined ventricular output to a permanently closed structure within a matter of hours or days (see section II.), it is predictable that the process of closure is associated with morphological changes. After birth, there is extensive remodeling of the vessel's wall, and this renders closure permanent. Even when patent, the structure of the ductus is quite different from the arteries it connects, being more muscular and relatively deficient in elastin. Furthermore, ductal elastin is less likely to be assembled into bundles of fibers compared with elastin in the aorta and pulmonary artery (Zhu et al., 1993).

The microscopic features of the patent fetal and closing neonatal ductus have been described in several species, including mice (Tada and Kishimoto, 1990), rats (Jarkovska et al., 1989), guinea pigs (Fay and Cooke, 1972), rabbits (Giuriato et al., 1993), dogs (Gittenberger de Groot et al., 1985), and humans (Silver et al., 1981; Tada et al., 1985). Closure is associated with the formation of intimal cushions, which are characterized by (a) an area of subendothelial edema, (b) infolding and ingrowth of endothelial cells, and (c) migration into the subendothelial space of undifferentiated medial smooth

muscle cells (Gittenberger de Groot et al., 1985). Postnatal remodeling is also associated with the disassembly of the internal elastic lamina, and loss of elastin may promote smooth muscle cell migration (de Reeder et al., 1990). Some of these changes begin about halfway through gestation in humans but are much more marked after functional closure of the ductus in the neonate (Slomp et al., 1997). Ductal remodeling may depend on ischemia of the vessel wall (Clyman et al., 1997a), but the loss of medial smooth muscle cells is by apoptosis rather than necrosis (Clyman et al., 1997a; Slomp et al., 1997).

The cellular processes underlying the formation of intimal cushions have been studied in some detail and have been recently reviewed (Rabinovitch, 1996). These involve complex interactions between growth factors (notably transforming growth factor-β), glycosaminoglycans (e.g. hyaluronan and chondroitin sulfate), fibronectin, and cell surface molecules (e.g. integrins).

B. Pharmacological Aspects of Remodeling

Indomethacin-induced contraction of the fetal ductus arteriosus is associated with similar morphological changes as occur after delivery (Okada et al., 1994), and the changes that normally occur in the neonate can be prevented by the administration of exogenous PGE$_2$ (Jarkovska et al., 1992). It is unlikely that these findings are indicative of a specific effect of PGE$_2$ because maintaining ductal patency by keeping the neonate in an anaerobic environment also prevented remodeling (Fay and Cooke, 1972). Paradoxically, studies on the effect of dilator PGs on remodeling have suggested potential stimulatory roles. For instance, levels of PGI$_2$ synthase localized by immunohistochemistry in the wall of the ductus were greatest in medial smooth muscle cells at the site of intimal cushions (de Reeder et al., 1989); the ductus of dogs with genetic predisposition toward PDA had reduced expression of PGI$_2$ synthase in their medial smooth muscle cells (de Reeder et al., 1989); and indomethacin inhibited the migration of ductal smooth muscle cells in an in vitro model (Koppel and Rabinovitch, 1993). Interestingly, smooth muscle cells obstructing the lumen of the closed neonatal mouse ductus still express mRNA, encoding the prostanoid EP$_4$ receptor (Nguyen et al, 1997). These findings are at present hard to reconcile with the effects of indomethacin in vivo.

Postnatal contraction of the ductus affects its response to vasoconstrictors and vasodilators. Greater degrees of contraction in vivo were associated with reduced sensitivity and maximum response of the isolated lamb ductus to PGE$_2$ but also reduced contraction in response to indomethacin and oxygen tension in vitro (Clyman et al., 1983a). Similarly, in the guinea pig neonate, progressive contraction of the ductus was associated with reduced dilation in response to anoxia and papaverine and reduced contraction in response to oxygen, acetylcholine, and potassium (Fay and Cooke, 1972). There

was a correlation between this generalized loss of responsiveness to vasoactive agents and the anatomical changes that occurred after birth. Furthermore, prevention of closure by keeping neonatal guinea pigs in an anaerobic environment maintained ductal responsiveness to variations in oxygen tension (Fay and Cooke, 1972). Similarly, when closure of the ductus in the neonatal lamb was prevented by occluding the pulmonary arteries, the isolated neonatal ductus in vitro retained a greater contractile response to indomethacin and oxygen and a greater dilator response to PGE$_2$ when compared with the ductus from appropriate controls (Clyman et al., 1989a). These findings suggest that remodeling of the vessel wall after birth alters the ability of endogenous and exogenous vasoactive agents to affect ductal patency. These changes can be stimulated and inhibited by diverse procedures promoting contraction and relaxation, respectively and, therefore, would appear to be a function of the degree of contraction of the ductus rather than any specific vasoactive agent, such as PGs.

The factors that control remodeling appear to mature with advancing gestational age. The ductus of the preterm human can reopen even after flow has been completely abolished by indomethacin-induced contraction (Weiss et al., 1995); this implies a failure of the remodeling process that renders closure irreversible. There is evidence in the preterm lamb to support this interpretation. The degree of postnatal contraction in vivo was correlated with the responsiveness of the isolated vessel in vitro, and it was found that for a given degree of ductal constriction in the neonate, the preterm vessel retained a greater degree of responsiveness to both contractile and dilator drugs (Clyman et al., 1985); this implies that the ability of contraction to induce remodeling is impaired in the preterm animal.

There is a recent preliminary report that complete contraction of the preterm ductus does not induce medial hypoxia as occurs in the term vessel and that this is associated with the failure of apoptosis of medial smooth muscle cells (Clyman et al., 1997a). The outer half on the media of the ductus is penetrated by vasa vasorum, and the neonatal vessel of the human has been noted to be hyperemic (Silver et al., 1981). Given that oxygen has a central role in promoting ductal contraction, it is possible that dilatation of the vasa vasorum may promote contraction of the ductus in the early stages of closure; this would necessitate different control mechanisms of the vasa vasorum smooth muscle and the smooth muscle of the ductus. The high levels of eNOS in the vasa vasorum (Clyman et al., 1997b) and the relative insensitivity of ductal smooth muscle to NO donors (Smith and McGrath 1993; Fox et al., 1996) might be explained by a requirement for differential control of these two smooth muscles in the process of ductal closure. However, this model is complicated by the potential role of medial hypoxia to promote remodeling (Clyman et al., 1997a). The role of the ductal vasa vasorum in postnatal

contraction is likely to be complex and is not yet fully resolved.

VIII. Integrated Model of Postnatal Ductal Contraction

The extensive literature on the physiology and pharmacology of the ductus can be reduced to a fairly simple model for ductal patency in utero and for contraction of the vessel after birth. All the following statements are referenced in the appropriate sections above. The main factors maintaining patency of the ductus in utero are low oxygen tension, high levels of circulating PGE_2, and locally produced PGE_2 and PGI_2. Fetal oxygen tension maintains the membrane potential of the vessel's smooth muscle in a hyperpolarized state, which inhibits the influx of calcium into ductal smooth muscle cells. Circulating and locally released PGs acting through the G-protein-coupled EP_4 and IP receptors maintain high levels of intracellular cAMP that depress the sensitivity of the contractile proteins of the ductus to calcium. The combined effects of these two pathways render the ductus virtually unresponsive to even massive stimulation, such as high micromolar concentrations of norepinephrine. After birth, oxygen tension rises and depolarizes ductal smooth muscle (by closure of delayed rectifier potassium channels) and results in an influx of calcium through L-type calcium channels. Furthermore, circulating levels of PGE_2 fall by an order of magnitude within hours of birth; levels of cAMP in ductal smooth muscle cells fall, releasing the "brake" on the responsiveness of the contractile proteins to calcium. This responsiveness is either intrinsically high or may be stimulated by elevated oxygen tension. These changes promote ductal contraction directly, decrease its sensitivity to vasodilators, and increase its sensitivity to a diverse range of vasoconstrictors. Contraction of the ductus then stimulates remodeling that renders closure irreversible.

IX. Clinical Significance

The clinical importance of the ductus is discussed simply to allow appreciation of the potential for novel therapies. Details of clinical management and therapeutic regimes have been reviewed elsewhere (Gersony, 1986; Van den Veyver and Moise, 1993; Hammerman, 1995).
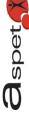
A. Patent Ductus Arteriosis

As the name suggests, PDA is failure of the ductus to close after birth. PDA is a relatively common complication of prematurity. Originally, the only option for treatment was surgical ligation of the ductus. The disadvantages of this in the context of prematurity were (*a*) the infants are small and often unwell (*b*) the required level of surgical expertise was only found in major centers, necessitating transfer of sick premature infants. The observation that indomethacin contracted the ductus arteriosus of lambs (Coceani et al., 1975) and rats

(Sharpe et al., 1974) led directly to its evaluation in human premature infants with PDA, and it is currently a widely used technique in the treatment of this condition (Gersony, 1986; Hammerman, 1995). The major adverse effects of indomethacin in the treatment of PDA are similar to the adult: renal impairment, gastrointestinal bleeding, and bleeding tendency (secondary to its effects on platelets). The association between PDA and prematurity is probably secondary to delivery occurring before the adaptations that occur in the ductus toward term that promote postnatal contraction (see section V). However, PDA is also associated with coexisting conditions of prematurity, such as respiratory distress syndrome, which itself is associated with increased circulating PG concentrations (see Hammerman, 1995 for review). PDA can also occur in the absence of prematurity, and, in such cases, the management is purely surgical.

Paradoxically, antenatal administration of indomethacin to the mother is associated with an increased risk of PDA (62% versus 44% in nonexposed) and an increased resort to surgical treatment of PDA (50% versus 20% in non exposed), even when obvious confounding variables are taken into account (Norton et al., 1993). There are reports of contraction of the ductus after maternal administration of corticosteroids (Azancot-Benisty et al., 1995), although this does not appear to be a common adverse effect (Eronen et al., 1993). A randomized controlled trial has demonstrated that antenatal administration of corticosteroids to the mother reduces the incidence of PDA in human infants born before 30 weeks gestation (Eronen et al., 1993).

B. Ductus-Dependent Circulation

The term "ductus-dependent circulation" applies to a range of congenital heart defects where, as a consequence of the defect, continued patency of the ductus arteriosus of the neonate is necessary for its survival (Freed et al., 1981; Gersony, 1986). There are 3 roles that the ductus can have in such circumstances: (*a*) to maintain adequate pulmonary blood flow, e.g. in pulmonary atresia; (*b*) to maintain adequate systemic blood flow, e.g. in aortic arch abnormalities; and (*c*) to improve mixing of the systemic and pulmonary circulations, e.g. in transposition of the great arteries. The natural history of these conditions is that the infant is often reasonably healthy at birth but then deteriorates in early neonatal life because the ductus closes. Formerly, detection of such a defect was an indication for emergency surgery before ductal closure was complete. The observation that PGE_2 was a potent dilator of the lamb ductus led directly to the use of PGE_1 (or PGE_2) to maintain artificially ductal patency until definitive surgery could be performed. It is not effective, however, when closure of the ductus is complete, but is most effective where the initial degree of closure is smallest (Clyman et al., 1983a). Intravenous PGE_1 has major adverse effects,

including cardiac arrest, apnea, seizures, pyrexia, hypotension, flushing, and diarrhea (Gersony, 1986).

C. Prostaglandin H Synthase Inhibitors and Pregnancy

There are 3 main situations where PGHS inhibitors are used in pregnant women (see Van den Veyver and Moise, 1993 for review): (*a*) treatment of preexisting medical disease, e.g. rheumatoid arthritis—generally, the PGHS inhibitor would be substituted for a simple analgesic for the duration of pregnancy (*b*) treatment of premature labor—PGHS inhibitors (indomethacin is most widely used) are uterine tocolytics; and (*c*) treatment of polyhydramnios (excessive liquor)—PGHS inhibitors (again, indomethacin is most widely used) decrease fetal urine output and this decreases production of liquor. One of the problems with these drugs in pregnancy is that they cross the placenta and contract the fetal ductus arteriosus. A dosage of 10 mg/kg indomethacin to the pregnant rat caused 70% constriction of the ductus of its fetuses and led to signs of right-sided heart failure in 1 to 8 h and right ventricular hypertrophy within 24 h (Momma and Takao, 1989b). When indomethacin was used in the treatment of the preterm labor in the human, with dosages of about 1 to 2 mg/kg, significant contraction of the ductus arteriosus was seen in over half of the fetuses, and the extent of contraction varied directly with the gestational age of the fetus (Moise et al., 1988). In another study, one-third of fetuses with ductal contraction secondary to therapeutic dosages of indomethacin had tricuspid regurgitation and, in one fetus (out of nine with ductal contraction), there was evidence of right-sided heart failure (Eronen et al., 1991).

Prenatal closure of the ductus in animals is associated with changes in the pulmonary circulation that promote pulmonary hypertension, e.g. after surgical ligation of the fetal lamb ductus (Shaul et al., 1997) or administration of indomethacin (1 mg/kg) to pregnant rats (Herget et al, 1993). Clinical trials of short term treatment with indomethacin have failed to demonstrate any major cardiovascular sequelae in human newborns exposed to the drug in utero, although pulmonary hypertension may be a problem with prolonged administration of PGHS inhibitors to pregnant women (see Van den Veyver and Moise, 1993).

X. Experimental Models for Novel Therapeutics

Elucidating the role of PGs in ductal control led directly to the therapies described above for the manipulation of the vessel's patency in the human neonate. As new potential therapies are identified, the details of the experimental basis for their evaluation against standard treatments will be critical. Taking the example of an agonist that relaxes the ductus, the evidence that it may be useful to maintain ductal patency in the human neonate could take the form of (*a*) its effect on the isolated ductus in vitro, (*b*) its effect on the ductus in the fetus with or without precontraction, and/or (*c*) its effect on closure of the ductus in the neonate. Studies in the neonate might include situations where other cardiovascular parameters could not be measured, e.g. when using a fetal or neonatal rat and evaluating ductal patency by microscopy of the frozen animal or where the effect of a given drug on other aspects of the animal's cardiovascular physiology could be established, e.g. in an instrumented neonatal lamb. Even in the simplest model, isolated ring preparations of the ductus, contradictory findings are obtained. For instance, one study found that SNP had a much greater effect on the isolated ductus than PGE_2 (Walsh and Mentzer, 1987), whereas another found that PGE_2 had a much greater effect than SNP (Smith and McGrath, 1993). These anomalies present a dilemma for clinicians planning clinical trials in human infants, but such contradictory findings can be reconciled by appreciating technical differences in experimental models.

A. In Vitro Comparisons of Drugs

It is standard to precontract an isolated vessel before obtaining a relaxation response (i.e., quantifying functional antagonism). In the case of the ductus arteriosus, several different methods have been described, including elevated oxygen tension, indomethacin, and norepinephrine. These techniques are often used in a variety of combinations. It has been observed, however, that these interventions can alter the relative potency of dilator agonists (Smith and McGrath, 1993, 1994), and this may explain many apparently anomalous and contradictory results.

The main confounding variable is the use of a PGHS inhibitor (e.g. indomethacin) to precontract the ductus. Indomethacin contracts the isolated ductus by removing the dilator effect of locally released PGs (Clyman et al., 1980a) and is one of the most common methods of precontracting the isolated ductus. However, indomethacin has different effects on the potencies of different agonists. Indomethacin increased the sensitivity of the ductus to exogenous PGE_2 (Coceani et al., 1975; Smith and McGrath, 1994). The effects of locally released PG in the isolated fetal rabbit ductus in the absence of indomethacin (as assessed by the sensitivity of the vessel to norepinephrine) was equivalent to approximately 0.5 to 1.0 nM exogenous PGE_2 in the presence of indomethacin (Smith and McGrath, 1994). It follows that in the absence of a PGHS inhibitor, exogenous PGE_2 will only have an effect on the ductus when it significantly augments or exceeds the concentration of endogenous PGE_2. In the absence of indomethacin, the threshold for an effect of exogenous PGE_2 was 1 nM, whereas in the presence of indomethacin, it was approximately 0.1 nM. Therefore, eliminating the effects of locally produced PGE_2 induced an apparent increase in the vessel's sensitivity to exogenous PGE_2 (Smith and McGrath, 1994). When the ductus was precontracted with 10 μM norepi-

nephrine, indomethacin decreased the sensitivity of the ductus to other vasodilators (cromakalim and forskolin) (Smith and McGrath, 1994); this effect was reversed by addition of subnanomolar concentrations of exogenous PGE_2. Therefore, indomethacin increased the sensitivity of the ductus to exogenous PGE_2 but decreased its sensitivity to other vasodilators, both effects resulting from the elimination of endogenous PGE_2 (Smith and McGrath, 1994).

In the study where PGE_2 was found to be more effective than SNP, indomethacin was used to precontract the vessel (Smith and McGrath, 1993), whereas in the other study it was not (Walsh and Mentzer, 1987). It is likely that in any comparison of prostanoid and nonprostanoid vasodilators in the ductus, the presence or absence of a PGHS inhibitor will have a major effect on the relative potencies of the drugs evaluated.

B. In Vivo Comparisons of Drugs in the Fetus

A comparison of drugs has been made in fetal lambs where the ductus was precontracted by indomethacin (Friedman et al., 1983) or by ventilation of the lungs with oxygen (Mentzer et al., 1985; Walsh et al., 1988). Comparing drugs in vivo, indomethacin might have an even greater effect on the relative potencies of PGE_2 and nonprostanoid vasodilators because of the contribution of circulating PGE_2. In the chronically instrumented lamb fetus, exogenous PGE_2 had no effect on the ductus before indomethacin, but was the most potent dilator of the vessel precontracted with indomethacin (Friedman et al., 1983). In the ductus precontracted by ventilation with oxygen, SNP and nitroglycerin had a greater effect than PGE_2 (Mentzer et al., 1988).

The key question regarding the use of PGHS inhibitors to precontract the ductus when making comparisons between dilator agonists (both in vitro and in vivo) is whether this procedure is likely to represent a better or worse simulation of the human neonate with a ductus-dependent congenital heart defect. In human infants with ductus-dependent congenital heart disease receiving exogenous PGE_2, plasma concentrations of PGE_2 (i.e., the sum of endogenous and exogenous PGE_2) are in the subnanomolar range (Silove et al., 1981). This strongly suggests that the endogenous levels of PGE_2 (the sum of circulating and locally released PGE_2) to which the ductus is exposed in these infants are low and would tend to suggest that experiments where PGHS inhibitors are used for precontraction are likely to be a better model for the therapeutic situation.

The best method for examining the effect of maternal anti-PG therapy on the fetal ductus is probably the one described by Friedman and colleagues (1983), which used piezoelectric crystals glued to the ductal adventitia to assess ductal contraction and fetal vascular catheters to assess hemodynamic changes in the chronically instrumented lamb fetus. Using this method, these authors examined the response of the in vivo fetal ductus

to a range of drugs and were able to quantify dose effect relationships. The ability to administer the drugs directly to the fetus circumvented any shortcomings related to differences in placental transfer comparing the sheep and the human.

C. In Vivo Comparisons of Drugs in the Neonate

From the foregoing, it would seem only logical that the best model of neonatal closure of the ductus would be a neonatal animal. There are a large number of studies looking at the effect of various drugs on ductal closure in neonatal rats using the whole-body freezing technique. The mother, fetus, or neonate is treated with a drug and then the whole fetus or neonate is frozen, sectioned, and ductal diameter is quantified using microscopy. This technique has the major disadvantage that it gives no information about the effect of a given drug on basic hemodynamic variables, such as blood pressure and heart rate. For instance, it has been demonstrated that propranolol delays ductal closure in this model (Arishima et al., 1995); this seems paradoxical given that propranolol has a contractile effect on the ductus (Bodach et al., 1980). One would expect propranolol to have many major effects on the physiology of the neonate, on blood pressure, on heart rate, on the activity of sympathetic pressor nerves, and on the renin-angiotensin system, for instance. It seems likely that the paradoxical effect of propranolol to delay closure in the rat pup is secondary to such a mechanism, but by the nature of the experiments there is no information to consider these possibilities.

The comparison of prostanoid and nonprostanoid ductal dilators in instrumented neonates should be an area for future study. Probably the best method described so far involves delivering the lamb by Cesarean section and instrumenting it while it is still connected to the umbilico-placental circulation. The neonate is then intubated and ventilated and the degree of ductal shunt quantified by measurement of the cardiac output distribution using radio-labeled microspheres. This has principally been used to assess ductal dilators (e.g. Clyman et al., 1983b). Theoretically, the same technique may be applied to the study of PGHS inhibitors to close the ductus. The use of a preterm rather than a term neonate would make it a better model of the clinical situation and would result in delayed physiological closure, allowing time to examine drug effects. The use of microspheres might be replaced by color-flow Doppler ultrasound, which would allow continuous assessment of shunt direction and magnitude and the degree of contraction.

XI. Scope for Novel Therapies

The key to manipulating the ductus arteriosus in the neonate is to affect the given change in ductal contractility with the minimum effect on systemic systems, particularly other smooth muscle. It follows, therefore,

that therapeutic strategies should concentrate on the aspects of smooth muscle control that are peculiar to the ductus. All current therapies are based around synthetic PGs to dilate the ductus and PGHS inhibitors to close it. Recent developments offer the potential for much more specific manipulation of the ductal PG system, but also indicate potential strategies acting on some non-PG control systems regulating its patency.

Several of the proposed therapies relate to drugs with specific activity at prostanoid receptor types and subtypes. Relatively few such drugs are available at present (Coleman et al., 1994b). However, the genes encoding these receptors have been cloned and sequenced (Abramovitz et al., 1995), and it is likely that many more such drugs will become available in the foreseeable future.

A. Patent Ductus Arteriosus

1. Prostanoid EP_4 receptor antagonist. Assuming that the EP receptors on the human and rabbit ductus arteriosus are the same, it has been proposed that an EP_4 receptor antagonist would be a more effective and less toxic treatment for PDA than indomethacin (Smith et al., 1994). Although this drug would not eliminate the effect of PGI_2 directly, it may well do so indirectly by removing potentiation of the effect of PGI_2 by endogenous PGE_2 (Smith and McGrath, 1994). Furthermore, it would preserve the contractile effects of TP and EP_3 stimulation, which will be eliminated by indomethacin (Smith and McGrath, 1995).

The expression of the human EP_4 receptor has been screened in several tissues using a complementary deoxyribonucleic acid probe from the cloned gene and Northern blot (this was published as the EP_2 receptor but was subsequently found to be EP_4). EP_4 receptor expression was found in many key tissues, including the heart, lung, small intestine, thymus, and kidney (Honda et al., 1993; Bastien et al., 1994; Nishigaki et al., 1995). The assumption that a selective prostanoid receptor antagonist is likely to have fewer side effects than a PGHS inhibitor is potentially flawed. Many tissues, as is the case in the ductus, have a mixed population of EP receptors, mediating opposing effects (Coleman et al., 1990). The same logic that predicts a greater therapeutic effectiveness of a selective receptor blocker might also predict adverse effects not experienced with PGHS inhibitors. In a tissue that expressed two EP subtypes with opposing actions, an overall loss of PGE_2 (as would occur with a PGHS inhibitor) may have a minimal effect when stimulation of the two receptors was balanced, whereas selective loss of the effects of one of the subtypes might be expected to have a greater effect. No potent, selective EP antagonists have yet been described, but it will be interesting to compare the adverse effects of these drugs with PGHS-1 and PGHS-2 inhibitors, as selective drugs of both types become available.

2. Isoform specific prostaglandin H synthase inhibitor. As discussed in section III.A.6., a recent preliminary report has identified that the main PGHS isoform in the neonatal ductus is PGHS-2 (Guerguerian et al., 1997). If the effect of indomethacin on the human infant with PDA is mediated in part by inhibiting PGHS in the wall of the ductus, there may be scope for the use of selective PGHS-2 inhibitors in the management of PDA. However, it has been demonstrated that infants with PDA have higher circulating concentrations of PGE_2 than controls (Lucas and Mitchell, 1978). If circulating PGE_2 is a major factor in the pathogenesis of PDA, then the efficacy of PGHS-2 inhibitors will depend on the PGHS isoform responsible for producing PGs systemically. A recent preliminary report demonstrated that a moderately selective PGHS-2 inhibitor, DuP697, had no effect on circulating PGE_2 or ductal patency in the neonatal lamb, whereas indomethacin decreased circulating PGE_2 and induced ductal closure. If this finding is confirmed with more selective PGHS-2 inhibitors (see section III.A.7.), there may be a role for the use of a selective PGHS-1 inhibitor to treat PDA. Although such a drug has been described (Jang et al., 1997), it is likely to have many of the major adverse effects of current nonselective PGHS inhibitors. It is also possible that both isoforms of PGHS produce precursors for PGE_2 in the preterm neonate and that nonselective drugs such as indomethacin will prove to be more effective than selective inhibitors of either isoform.

3. Potassium channel closing agents. Various potassium channel blockers have been described (Kuriyama et al., 1995), and, given the probable role of the delayed rectifier potassium channel in mediating the oxygen-induced contraction of the ductus (Tristani-Firouzi et al., 1996), there is at least the theoretical possibility of the use of such a drug in PDA. However, drug treatment of PDA is only performed in premature infants (Gersony, 1986). Because the oxygen-induced contraction is poorly developed in the preterm animal (see section V.), attempts to use this pathway to promote contraction may be less likely to be successful than the strategy of blocking the potent dilator systems of the preterm ductus.

B. Ductus-Dependent Circulation

There are several general points about improving the effectiveness of drug treatment in ductus-dependent circulation. First, there is evidence to suggest that the effectiveness of PGE_2 in maintaining ductal patency in the neonate is increased with lesser degrees of initial constriction that is probably related to remodeling of the vessel as it contracts (Clyman et al., 1983a). The effectiveness of current therapy (intravenous PGE_1), therefore, could be improved by screening antenatally for ductus-dependent congenital heart lesions using fetal echocardiography and commencing PGE_1 infusion immediately after delivery (Smith, 1992).

Second, the inhibitory effect of increased oxygen tension on the response of the ductus to PGE_2 (Coceani et al., 1975; Smith and McGrath, 1991, 1993) has implications for the treatment of ductus-dependent circulation. In cyanotic congenital heart disease, PGE_1 increases arterial oxygen tension (Freed et al., 1981), i.e., the beneficial effect of PGE_1 inhibits the sensitivity of its target. In such cases, PGE_1 may well be self-limiting. However, this is likely to be a drawback with all potential dilator therapies because oxygen has the same effect on a range of vasodilators with diverse mechanisms of action (Smith and McGrath, 1993). A dilator that had a specific effect on the mechanism of the oxygen-induced contraction may not have this drawback, but no such agent has yet been described.

1. Prostanoid EP_4 receptor agonist. Assuming that the EP receptors on human and rabbit ductus are the same, a selective EP_4 agonist should be effective in maintaining ductal patency in ductus-dependent circulation (Smith et al., 1994). First, it would be expected to have fewer side effects than PGE_1 because it would not stimulate EP_1, EP_2, or EP_3 receptors, which are ubiquitously expressed (Coleman et al., 1990; Abramovitz et al., 1995). Second, it may be more potent than PGE_1 because it would not stimulate contractile EP_3 receptors on the ductus (Smith and McGrath, 1995). As with the EP_4 antagonist, the tendency of tissues to have balanced populations of EP receptors (i.e., different subtypes with opposing effects) raises the possibility of adverse effects with a selective agonist that would not have been predicted from experience with PGE_1 or PGE_2. Nevertheless, a selective EP_4 agonist may well be one of the most promising avenues for drug development for manipulating ductal patency.

2. Potassium channel activators. Because membrane potential is clearly central in the control of closure of the ductus (see sections IV.A.4. and VI.A.), drugs acting at potassium channels are strong potential candidates in ductus-dependent circulation. In the absence of indomethacin, the isolated ductus was more sensitive to the ATP sensitive potassium channel activator, cromakalim, than other blood vessels (see section VI.) and, therefore, cromaklin or related drugs may be relatively selective for the ductus (Smith and McGrath, 1994). At present, there are no studies of the effect of these drugs on ductal closure in the neonate.

3. Nitric oxide donors. Again, NO donors have been shown to be potent dilators of the fetal ductus in vivo (Walsh et al., 1988) and the isolated ductus in vitro (Walsh and Mentzer, 1987) in the absence of indomethacin. The current evidence about their relative potency compared with PGE_2 is simply insufficient to predict how they might perform in the human neonate, for the reasons described in section X. The relative effectiveness of NO donors and PGE_2 in maintaining ductal patency for a given degree of adverse effects needs to be com-

pared in a realistic animal model of ductal closure in the neonate.

4. ET_A receptor antagonist. Given that ET-1 acting through the ET_A receptor was first proposed to mediate the oxygen-induced contraction of the ductus 5 years ago (Coceani et al., 1992), it is perhaps surprising that there have been no reports of the effect of ET_A receptor antagonists on ductal closure in the neonate. These drugs are readily available and their effect on the ductus in the intact animal should be evaluated. Given the ubiquitous role of the ET_A receptor in cardiovascular homeostasis (Masaki et al., 1994), however, even if they have an effect on the ductus, their clinical usefulness may be limited by systemic cardiovascular effects.

C. Preterm Labor

1. Prostanoid EP_3/FP receptor antagonists. The main prostanoid receptors mediating contraction in myometrial strips from pregnant women are EP_3, FP, and TP (Senior et al., 1993). Given the key role postulated for PGE_2 in the control of parturition (see Drife and Calder, 1992 for review), there is scope for the development of a selective EP_3 receptor antagonist as a uterine tocolytic. An EP_3 receptor antagonist would have the advantage over all PGHS inhibitors (selective or otherwise, see Section XI.C.2.) of maintaining the inhibitory effects on myometrial contractility of PGI_2 and PGE_2 acting through the IP and EP_2 receptors, respectively (Senior et al., 1993). Neither an EP_3 nor an FP receptor antagonist would be expected to contract the fetal ductus arteriosus even if they did cross the placenta (Smith et al., 1994).

2. Prostaglandin H synthase-2 inhibitors. A more immediately available option in the treatment of preterm labor is the use of selective blockers of PGHS-2. Several drugs have been described with greater than a thousand-fold selectivity for PGHS-2 over PGHS-1 (Riendeau et al., 1997). As described in section III, PGHS-1 appears to be the major isoform producing precursors for PGE_2 to maintain ductal patency in utero (Guerguerian et al., 1997). Preliminary observations from a single uncontrolled trial of nimesulide (about ninety-fold selective for PGHS-2 over PGHS-1 [Riendeau et al., 1997]) in pregnancy found that maternal administration of this drug between 18 and 34 weeks gestation had no effect on Doppler ultrasound waveforms in the human fetal ductus (Sawdy et al., 1997). Interestingly, PGHS-2 may have a role in the control of perfusion of the human fetal kidney (Komhoff et al, 1997). The relative efficacy of PGHS-2 inhibitors in the treatment of preterm labor and confirmation of their lack of effect on the fetal ductus will require randomized comparisons with indomethacin or one of the other nonselective PGHS inhibitors.

3. Sulindac. Another option in terms of PGHS inhibition is a drug that does not cross the placenta. Indomethacin, the most widely used PGHS inhibitor in the management of preterm labor, freely crosses the human

placenta, with a fetal to maternal concentration ratio of 0.97 (Moise et al., 1990). Although sulindac freely crosses the human placenta, maternal levels of its active metabolite are higher than fetal levels (Kramer et al., 1995) and it has been shown in controlled trials to have less of an effect on the ductus than indomethacin (Carlan et al., 1992; Rasanen and Jouppila, 1995). Sulindac is seven-fold more potent at PGHS-2 than PGHS-1 (Riendeau et al., 1997), which may also explain its lesser effect on the ductus. Although these findings are promising, large scale trials will be required to establish that the efficacy of sulindac is similar to indomethacin, given the unpredictable course of apparent preterm labor.

XII. Conclusions

(*a*) The key factors relaxing ductal smooth muscle cells in utero are the synergistic dilator effects of (1) locally released and circulating PGE_2, acting through prostanoid EP_4 receptors, positively coupled to AC, producing cAMP that reduces the sensitivity of the contractile proteins to calcium (and may alter $[Ca^{2+}]_i$) and (2) fetal oxygen tension keeping potassium channels open, hyperpolarizing the membrane and inhibiting calcium influx. These factors potentiate the dilator effect of several other inhibitory systems, including NO.

(*b*) At birth, increasing oxygen tension closes the delayed rectifier potassium channels and depolarizes the membrane and calcium enters the ductal smooth muscle cells through L-type channels. The sensitivity of the contractile proteins is high (either intrinsically or stimulated by elevated oxygen tension) and inhibition of this profound sensitivity to calcium by PGE_2 is lost. The combination of calcium influx and increased sensitivity of the contractile proteins to calcium result in a profound contractile response, an insensitivity to vasodilators, and a profound increased sensitivity to vasoconstrictors.

(*c*) Exogenous PGE_2 maintains ductal patency in the neonate, and PGHS inhibitors close the fetal and the neonatal ductus. There is the clear potential for the development of highly selective drugs to manipulate ductal patency in the sick human neonate, particularly selective prostanoid EP_4 receptor agonists or antagonists, as appropriate, and/or drugs with activity at potassium channels.

(*d*) Selective inhibitors of PGHS-2 or selective non-EP_4 prostanoid receptor antagonists should allow maternal anti-PG therapy without contracting the fetal ductus arteriosus.

(*e*) In vitro and in utero comparisons of drugs may be profoundly affected by the method of precontraction employed, in particular the presence or absence of indomethacin. Better animal models of neonatal closure need to be developed to test novel drugs as they become available.

REFERENCES

Abramovitz M, Adam M, Boie Y, Grygorczyk R, Rushmore TH, Nguyen T, Funk CD, Bastien L, Sawyer N, Rochette C, Slipetz DM and Metters KM (1995) Human prostanoid receptors: Cloning and characterization. *Adv Prostaglandin Thromboxane Leukotriene Res* **23:**499–504.

Anderson DF, Bissonnette JM, Faber JJ and Thornburg KL (1981) Central shunt flows and pressures in the mature fetal lamb. *Am J Physiol* **241:**H415–H420.

Arishima K, Takizawa T, Oda T, Yamamoto M, Togashi H, Somiya H and Eguchi Y (1995) Propranolol inhibits the spontaneous closure of the ductus arteriosus in newborn rats. *J Vet Med Sci* **57:**943–944.

Aronson S, Gennser G, Owman CH and Sjoberg NO (1970) Innervation and contractile response of human ductus arteriosus. *Eur J Pharmacol* **11:**178–186.

Azancot-Benisty A, Benifla JL, Matias A, De-Crepy A and Madelenat P (1995) Constriction of the fetal ductus arteriosus during prenatal betamethasone therapy. *Obstet Gynecol* **85:**874–876.

Ball EG, Strittmatter CF and Cooper O (1951) The reaction of cytochrome oxidase with carbon monoxide. *J Biol Chem* **193:**635–647.

Bastien L, Sawyer N, Grygorczy KR, Metters KM and Adam M (1994) Cloning functional expression and characterization of the human prostaglandin E_2 receptor EP_2 subtype. *J Biol Chem* **269:**11873–11877.

Beech DJ, Zhang H, Nakao K and Bolton TB (1993) Potassium channel activation by nucleotide diphosphates and its inhibition by glibenclamide in vascular smooth muscle cells. *Br J Pharmacol* **110:**573–582.

Bodach E, Coceani F, Dumbrille A, Okpako DT and Olley PM (1980) The response of the isolated ductus arteriosus to transmural stimulation and drugs. *Br J Pharmacol* **71:**419–427.

Boie Y, Rushmore TH, Darmon-Goodwin A, Grygorczyk R, Slipetz DM, Metters KM and Abramovitz M (1994) Cloning and expression of a cDNA for the human prostanoid IP receptor. *J Biol Chem* **269:**12173–12178.

Boreus LO, McMurphy DM and Olson L (1969) Demonstration of adrenergic receptor function and innervation in the ductus arteriosus of the human fetus. *Acta Physiol Scand* **77:**316–321.

Born GVR, Dawes GS, Mott JC and Rennick BR (1955) The relief of central cyanosis caused by pulmonary arterio-venous shunts by creation of an artificial ductus arteriosus. *J Physiol* **130:**167–190.

Boyd JD (1941) The nerve supply of the mammalian ductus arteriosus. *J Anat* **75:**457–468.

Bray KM, Weston AH, Duty S, Newgreen DT, Longmore J, Edwards G and Brown TJ (1991) Differences between the effects of cromakalim and nifedipine on agonist-induced responses in rabbit aorta. *Br J Pharmacol* **102:**337–344.

Bustamante SA, Pang Y, Romero S, Pierce MR, Voelker CA, Thompson JH, Sandoval M, Liu X and Miller MJS. (1996) Inducible nitric oxide synthase and the regulation of central vessel caliber in the fetal lamb. *Circulation* **94:**1948–1953.

Carlan SJ, O'Brien WF, O'Leary TD and Mastrogiannis D (1992) Randomized comparative trial of indomethacin and sulindac for the treatment of refractory preterm labor. *Obstet Gynecol* **79:**223–228.

Chang SW, Dutton D, Wang HL, He LS, Sterns R, Hui A, Giacomini KM, Ortiz De Montellano P and Voelkel NF (1992) Intact lung cytochrome P_{450} is not required for hypoxic pulmonary vasoconstriction. *Am J Physiol* **263:**L446–L453.

Chatfield BA, McMurty IF, Hall SL and Abman SH (1991) Hemodynamic effects of endothelin-1 on ovine fetal pulmonary circulation. *Am J Physiol* **261:**R182–R187.

Clyman RI (1987) Ductus arteriosus: Current theories of prenatal and postnatal regulation. *Semin Perinatol* **11:**64–71.

Clyman RI, Campbell D, Heymann MA and Mauray F (1985) Persistent responsiveness of the neonatal ductus arteriosus in immature lambs: A possible cause for reopening of patent ductus arteriosus after indomethacin-induced closure. *Circulation* **71:**141–145.

Clyman RI, Evans S and Koch C (1997a) Hypoxia initiates ductus arteriosus remodeling. *Pediatr Res* **41:**19A.

Clyman RI, Heymann MA and Rudolph AM (1977) Ductus arteriosus responses to prostaglandin E_1 at high and low oxygen concentrations. *Prostaglandins* **13:**219–223.

Clyman RI, Mauray F, Demers LM, Rudolph AM and Roman C (1979) Developmental responses to indomethacin: A comparison of isometric tension with PGE_2 formation in the lamb ductus arteriosus. *Prostaglandins* **18:**721–730.

Clyman RI, Mauray F, Demers LM, Rudolph AM and Roman C (1980a) Does oxygen regulate prostaglandin-induced relaxation in the lamb ductus arteriosus. *Prostaglandins* **19:**489–498.

Clyman RI, Mauray F, Heymann MA and Roman C (1987) Cardiovascular effects of patent ductus arteriosus in preterm lambs with respiratory distress. *J Pediatr* **111:**579–587.

Clyman RI, Mauray F, Heymann MA and Roman C (1989a) Influence of increased pulmonary vascular pressures on the closure of the ductus arteriosus in newborn lambs. *Pediatr Res* **25:**136–142.

Clyman RI, Mauray F, Heymann MA and Rudolph AM (1978a) Ductus arteriosus: Developmental response to oxygen and indomethacin. *Prostaglandins* **15:**993–998.

Clyman RI, Mauray F, Koerper MA, Wiemer F, Heymann MA and Rudolph AM (1978b) Formation of prostacyclin (PGI_2) by the ductus arteriosus of fetal lambs at different stages of gestation. *Prostaglandins* **16:**633–642.

Clyman RI, Mauray F, Roman C and Rudolph AM (1978c) PGE_2 is a more potent

dilator of the lamb ductus arteriosus than is either PGI_2 or 6 keto $PGF_{1\alpha}$ Prostaglandins. **16**:259–264.

Clyman RI, Mauray F, Roman C, Heymann MA and Payne B (1983a) Factors determining the loss of ductus arteriosus responsiveness to prostaglandin E_2. *Circulation* **68**:433–436.

Clyman RI, Mauray F, Roman C, Heymann MA and Payne B (1983b) Effect of gestational age on ductus arteriosus response to circulating prostaglandin E_2. *J Pediatr* **102**:907–911.

Clyman RI, Mauray F, Roman C, Heymann MA, Ballard PL, Rudolph AM and Payne B (1981a) Effects of antenatal glucocorticoid on ductus arteriosus of preterm lambs. *Am J Physiol* **241**:H415–H420.

Clyman RI, Mauray F, Roman C, Rudolph AM and Heymann MA (1980b) Circulating prostaglandin E_2 concentrations and patent ductus arteriosus in fetal and neonatal lambs. *J Pediatr* **97**:455–461.

Clyman RI, Mauray F, Roman C, Rudolph AM and Heymann MA (1981b) Glucocorticoids alter the sensitivity of the lamb ductus arteriosus to prostaglandin E_2. *J Pediatr* **98**:126–128.

Clyman RI, Mauray F, Rudolph AM and Heymann MA (1980c) Age-dependent sensitivity of the lamb ductus arteriosus to indomethacin and prostaglandins. *J Pediatr* **96**:94–98.

Clyman RI, Saugstad OD and Mauray F (1989b) Reactive oxygen metabolites relax the lamb ductus arteriosus by stimulating prostaglandin production. *Circ Res* **64**:1–8.

Clyman RI, Waleh N, Black S and Riemer RK (1997b) Nitric oxide (NO) vasa vasorum (vv) and the ductus arteriosus. *Pediatr Res* **41**:143A.

Clyman RI, Wong L, Heymann MA and Rudolph AM (1978d) Responsiveness of the lamb ductus arteriosus to prostaglandins and their metabolites. *Prostaglandins* **15**:325–331.

Coceani F (1994) Control of the ductus arteriosus - a new function for cytochrome P_{450} endothelin and nitric oxide. *Biochem Pharmacol* **48**:1315–1318.

Coceani F and Kelsey L (1991) Endothelin-1 release from lamb ductus arteriosus: Relevance to postnatal closure of the vessel. *Can J Physiol Pharmacol* **69**:218–221.

Coceani F and Olley PM (1973) The response of the ductus arteriosus to prostaglandins. *Can J Physiol Pharmacol* **51**:220–225.

Coceani F and Olley PM (1988) The control of cardiovascular shunts in the fetal and perinatal period. *Can J Physiol Pharmacol* **66**:1129–1134.

Coceani F, Armstrong C and Kelsey L (1989a) Endothelin is a potent constrictor of the lamb ductus arteriosus. *Can J Physiol Pharmacol* **67**:902–904.

Coceani F, Bishai I, White E, Bodach E and Olley PM (1978a) Action of prostaglandins endoperoxides and thromboxanes on the lamb ductus arteriosus. *Am J Physiol* **234**:H117–H122.

Coceani F, Bodach E, White E and Olley PM (1980) 6 keto-PGE_1 is a potent relaxant of the fetal ductus arteriosus. *Prostaglandins* **19**:109–113.

Coceani F, Bodach E, White E, Bishai I and Olley PM (1978b) Prostaglandin I_2 is less relaxant than prostaglandin E_2 on the lamb ductus arteriosus. *Prostaglandins* **15**:551–556.

Coceani F, Breen CA, Lees JG, Falck JR and Olley PM (1988) Further evidence implicating a cytochrome P_{450}-mediated reaction in the contractile tension of the lamb ductus arteriosus. *Circ Res* **62**:471–477.

Coceani F, Hamilton NC, Labuc J and Olley PM (1984) Cytochrome P_{450}-linked monooxygenase: Involvement in the lamb ductus arteriosus. *Am J Physiol* **246**:H640–H643.

Coceani F, Huhtanen D, Hamilton NC, Bishai I and Olley PM (1986) Involvement of intramural prostaglandin E_2 in prenatal patency of the lamb ductus arteriosus. *Can J Physio Pharmacol* **64**:737–744.

Coceani F, Jhamandas VM, Bodach E, Labuc J and Borgeat P (1982) Evidence against a role for lipoxygenase-derived products of arachidonic acid in the lamb ductus arteriosus. *Can J Physiol Pharmacol* **60**:345–349.

Coceani F, Kelsey L, Ackerly C, Rabinovitch M and Gelboin H (1994a) Cytochrome P_{450} during ontogenic development: Occurrence in the ductus arteriosus and other tissues. *Can J Physiol Pharmacol* **72**:217–226.

Coceani F, Kelsey L and Siedlitz E (1992) Evidence for an effector role of endothelin in closure of ductus arteriosus at birth. *Can J Physiol Pharmacol* **70**:1061–1064.

Coceani F, Kelsey L and Siedlitz E (1994b) Occurrence of endothelium-derived relaxing factor-nitric oxide in the lamb ductus arteriosus. *Can J Physiol Pharmacol* **72**:82–88.

Coceani F, Kelsey L and Seidlitz E (1996a) Carbon monoxide-induced relaxation of the ductus arteriosus in the lamb: Evidence against the prime role of guanylyl cyclase. *Br J Pharmacol* **118**:1689–1696.

Coceani F, Kelsey L, Seidlitz E and Korzekwa K (1996b) Inhibition of the contraction of the ductus arteriosus to oxygen by 1-aminobenzotriazole a mechanism-based inactivator of cytochrome P_{450}. *Br J Pharmacol* **117**:1586–1592.

Coceani F, Kelsey L, Seidlitz E, Marks GS, McLaughlin BE, Vreman HJ, Stevenson DK, Rabinovitch M and Ackerley C (1997) Carbon monoxide formation in the ductus arteriosus in the lamb: Implications for the regulation of muscle tone. *Br J Pharmacol* **120**:599–608.

Coceani F, Olley PM and Bodach E (1975) Lamb ductus arteriosus: Effect of prostaglandin synthesis inhibitors on the muscle tone and the response to prostaglandin E_2. *Prostaglandins* **9**:299–308.

Coceani F, White E, Bodach E and Olley PM (1979) Age-dependent changes in the response of the lamb ductus arteriosus to oxygen and ibuprofen. *Can J Physiol Pharmacol* **57**:825–831.

Coceani F, Wright J and Breen C (1989b) Ductus arteriosus involvement of a sarcolemmal cytochrome P_{450} in oxygen constriction. *Can J Physiol Pharmacol* **67**:1448–1450.

Colbert MC, Kirby ML and Robbins J (1996) Endogenous retinoic acid signaling colocalizes with advanced expression of the adult smooth muscle heavy chain isoform during development of the ductus arteriosus. *Circ Res* **78**:790–798.

Coleman RA, Grix SP, Head SA, Louttit JB, Mallett A and Sheldrick RLG (1994a) A novel inhibitory prostanoid receptor in piglet saphenous vein. *Prostaglandins* **47**:151–168.

Coleman RA, Kennedy I, Humphrey PPA, Bunce K and Lumley P (1990) Prostanoids and their receptors, in Comprehensive Medicinal Chemistry (Hansch C, Sammes PG and Taylor JB, eds) vol 3 pp 643–714, Pergamon Press, Oxford, UK.

Coleman RA, Smith WL and Narumiya S (1994b) The VIII International Union of Pharmacology classification of prostanoid receptors: Properties distribution and structure of the receptors and their sub-types. *Pharmacol Rev* **46**:205–229.

Crichton CA, Smith GCS and Smith GL (1997) Alpha-toxin-permeabilised rabbit fetal ductus arteriosus in more sensitive to Ca^{2+} than aorta or main pulmonary artery. *Cardiovasc Res* **33**:223–229.

Dawes GS, Mott JC and Widdicombe JG (1955) The patency of the ductus arteriosus in newborn lambs and its physiological consequences. *J Physiol* **128**:361–383.

de Reeder EG, Gittenberger-De Groot AC, Van Munsteren JC, Poelmann RE, Patterson DF and Keirse MJ (1989) Distribution of prostacyclin synthase 6-keto-prostaglandin F1 alpha and 15 hydroxy-prostaglandin dehydrogenase in the normal and persistent ductus arteriosus of the dog. *Am J Pathol* **135**:881–887.

de Reeder EG, Van-Munsteren CJ, Poelmann RE, Patterson DF and Gittenberger-De-Groot AC (1990) Changes in distribution of elastin and elastin receptor during intimal cushion formation in the ductus arteriosus. *Anat Embryol* **182**:473–480.

Dinchuk JE, Car BD, Focht RJ, Johnston JJ, Jaffee BD, Covington MB, Contel NR, Eng VM, Collins RJ, Czerniak PM, Gorry SA, Trzaskos JM (1995) Renal abnormalities and an altered inflammatory response in mice lacking cyclooxygenase II. *Nature* **378**:406–409.

Drayton MR and Skidmore R (1987) Ductus arteriosus blood flow during the first 48 h of life. *Arch Dis Child* **62**:1030–1034.

Drife JO and Calder AA (1992) Prostaglandins and the Uterus. Springer-Verlag, London.

Eronen M, Kari A, Pesonen E and Hallman M (1993) The effect of antenatal dexamethasone administration on the fetal and neonatal ductus arteriosus a randomized double-blind study. *Am J Dis Child* **147**:187–192.

Eronen M, Pesonen E, Kurki T, Ylikorkala O and Hallman M (1991) The effects of indomethacin and a β-sympathomimetic agent of the fetal ductus arteriosus during treatment of premature labor: A randomized double-blind study. *Am J Obstet Gynecol* **164**:141–146.

Farrugia G, Irons WA, Rae JL, Sarr MG and Szurszewski JH (1993) Activation of whole cell currents in isolated human jejunal circular smooth muscle cells by carbon monoxide. *Am J Physiol* **264**:G1184–G1189.

Fay FS (1971) Guinea pig ductus arteriosus I Cellular and metabolic basis for oxygen sensitivity. *Am J Physiol* **221**:470–479.

Fay FS and Cooke PH (1972) Guinea pig ductus arteriosus II Irreversible closure after birth. *Am J Physiol* **222**:841–849.

Fay FS and Jobsis FF (1972) Guinea pig ductus arteriosus III Light absorption changes during response to O2. *Am J Physiol* **223**:588–595.

Fox JJ, Ziegler JW, Ivy DD, Halbower AC, Kinsella JP and Abman SH (1996) Role of nitric oxide and cGMP system in regulation of ductus arteriosus tone in ovine fetus. *Am J Physiol* **271**:H2638–H2645.

Freed MD, Heymann MA, Lewis AB, Roehl SL and Kensey RC (1981) Prostaglandin E_1 in infants with ductus arteriosus-dependent congenital heart disease. *Circulation* **64**:899–905.

Friedman WF, Printz MP, Kirkpatrick SE and Hoskins EJ (1983) The vasoactivity of the fetal lamb ductus arteriosus studied in utero. *Pediatr Res* **17**:331–337.

Frölich JC (1997) A classification of NSAIDS according to the relative inhibition of cyclooxygenase isoenzymes. *Trends Pharmacol Sci* **18**:30–34.

Gersony WM (1986) Patent ductus arteriosus in the neonate. *Pediatr Clin North Am* **33**:545–560.

Gittenberger de Groot AC, Strengers JLM, Mentink M, Poelmann RE and Patterson DF (1985) Histologic studies on normal and persistent ductus arteriosus in the dog. *J Am Coll Cardiol* **6**:394–404.

Giuriato L, Scatena M, Chiavegato A, Guidolin D, Pauletto P and Sartore S (1993) Rabbit ductus arteriosus during development: Anatomical structure and smooth muscle cell composition. *Anat Rec* **235**:95–110.

Guerguerian AM, Hardy P, Lahaie I, Bhattacharya M, Peri K, Segar J, Olley P, Fouron JC, St-Louis J, Varma DR and Chemtob S (1997) Cyclooxygenase- (COX) 2 is the main intrinsic source of prostaglandins (PG) in the ductus arteriosus (DA) of the newborn (NB) but not of the fetus. *Pediatr Res* **41**:21A.

Hammerman C (1995) Patent ductus arteriosus: Clinical relevance of prostaglandins and prostaglandin inhibitors in PDA pathophysiology and treatment. *Clin Perinatol* **22**:457–479.

Hanssens M, Keirse MJN, Vankelecom F and Van Assche FA (1991) Fetal and neonatal effects of treatment with angiotensin-converting enzyme inhibitors in pregnancy. *Obstet Gynecol* **78**:128–133.

Harder DR, Campbell WB and Roman RJ (1995) Role of cytochrome P_{450} enzymes and metabolites of arachidonic acid in the control of vascular tone. *J Vasc Res* **32**:79–92.

Herget J, Hampl V, Povysilova V and Slavik Z (1993) Long-term effects of prenatal indomethacin administration on the pulmonary circulation in rats. *Eur Respir J* **8**:209–215.

Heymann MA and Rudolph AM (1975) Control of the ductus arteriosus. *Physiol Rev* **55**:62–78.

Heymann MA, Rudolph AM, Nies AS and Melmon KL (1969) Bradykinin production associated with oxygenation of the fetal lamb. *Circ Res* **25**:521–534.

Hobbs AJ, Fukuto JM and Ignarro LJ (1994) Formation of free nitric oxide from L-arginine by nitric oxide synthase: Direct enhancement of generation by superoxide dismutase. *Proc Natl Acad Sci USA* **91**:10992–10996.

Honda A, Sugimoto Y, Namba T, Watabe A, Irie A, Negishi M, Narumiya S and Ichikawa A (1993) Cloning and expression of a cDNA for mouse prostaglandin E receptor EP-2 subtype. *J Biol Chem* **68**:7759–7762.

Hornblad PY and Larsson KS (1972) Studies on the closure of the ductus arteriosus IX: Transitory effect of phenoxybenzamine on ductal closure in the guinea pig. *Acta Pharmacol Toxicol* **31**:149–152.

Ikeda M (1970) Adrenergic innervation of the ductus arteriosus of the fetal lamb. *Experientia* **26**:525–526.

Ikeda M, Rubenstein EH and Sonnenschein RR (1973a) Interaction of three factors in the closure of the ductus arteriosus. *Proc Soc Exp Biol Med* **143:**354–359.

Ikeda M, Rubenstein EH and Sonnenschein RR (1973b) Development of the oxygen-induced contraction in the ductus arteriosus of the guinea pig. *Experientia* **29:**445–446.

Ikeda M, Sonnenschein RR and Masuoka DT (1972) Catecholamine content and uptake of the ductus arteriosus of the fetal lamb. *Experientia* **28:**914–915.

Jang M, Cai L, Udeani GO, Slowing KV, Thomas CF, Beecher CWW, Fong HHS, Farnsworth NR, Kinghorn AD, Mehta RG, Moon RC and Pezzuto JM (1997) Cancer chemopreventive activity of resveratrol and natural product derived from grapes. *Science* **275:**218–220.

Jarkovska D, Janatova T, Hruda J, Ostadal B and Samanek M (1992) Effect of prostaglandin E_2 on the ductus arteriosus in the newborn rat an ultrastructural study. *Physiol Res* **41:**323–330.

Karaki H, Ozaki H, Hori M, Mitsui-Saito M, Amano K-I, Harada K-I, Miyamoto S, Nakazawa H, Won K-J and Koichi S (1997) Calcium movements distribution and functions in smooth muscle. *Pharmacol Rev* **49:**157–230.

Kennedy J and Clark SC (1942) Observations on the physiological reactions of the ductus arteriosus. *Am J Physiol* **136:**140–147.

Kim H-S, Aikawa M, Kimura K, Kuro-O M, Nakahara K-I, Suzuki T, Katoh H, Okamoto E-I, Yazaki Y and Nagai R (1993) Ductus arteriosus: Advanced differentiation of smooth muscle cells demonstrated by myosin heavy chain isoform expression in rabbits. *Circulation* **88:**1804–1810.

Komhoff M, Groene HJ, Klein T, Seyberth HW and Nuesing-R-M (1997) Localization of cyclooxygenase-1 and -2 in adult and fetal human kidney: Implication for renal function. *Am J Physiol* **272:**F460–F468.

Koppel R and Rabinovitch M (1993) Regulation of fetal lamb ductus arteriosus smooth muscle cell migration by indomethacin and dexamethasone. *Pediatr Res* **33:**352–358.

Kovalcik V (1963) The response of the isolated ductus arteriosus to oxygen and anoxia. *J Physiol* **169:**185–197.

Kramer WB, Saade G, Ou CN, Rognerud C, Dorman K, Mayes M and Moise KJ (1995) Placental transfer of sulindac and its active sulfide metabolite in humans. *Am J Obstet Gynecol* **172:**886–890.

Kriska M, Smiesko V, Cerletti C and Del Maschio A (1990) Interaction of prostaglandins and the myogenic factor in the mechanism of closure of the ductus arteriosus. *Physiol Bohemoslov* **39:**207–216.

Kuriyama H, Kitamura K and Nabata H (1995) Pharmacological and physiological significance of ion channels and factors that modulate them in vascular tissues. *Pharmacol Rev* **47:**387–573.

Langenbach R, Morham SG, Tiano HF, Loftin CD, Ghanayem BI, Chulada PC, Mahler JF, Lee CA, Goulding EH, Kluckman KD, Kim HS, Smithies O (1995) Prostaglandin synthase 1 gene disruption in mice reduces arachidonic acid-induced inflammation and indomethacin-induced gastric ulceration. *Cell* **83:**483–492.

Lucas A and Mitchell MD (1978) Plasma-prostaglandins in pre-term neonates before and after treatment for patent ductus arteriosus. *Lancet* **2:**130–132.

Lydrup ML, Sward K and Hellstrand P (1994) Effect of glibenclamide on membrane response to metabolic inhibition in smooth muscle of rat portal vein. *J Vas Res* **31:**82–91.

MacDonald AA, Poot P and Wensing CJG (1983) Nerve endings in the pulmonary trunk ductus arteriosus and aorta of intact and decapitated pig fetuses. *Anat Embryol* **168:**395–404.

Masaki T, Vane JR and Vanhoutte PM (1994) International union of pharmacology nomenclature of endothelin receptors. *Pharmacol Rev* **46:**137–142.

McGrath JC, MacLennan SJ, Mann AC, Stuart-Smith K and Whittle MJ (1986) Contraction of human umbilical artery but not vein by oxygen. *J Physiol* **380:**513–520.

McMurphy DM and Boreus LO (1971) Studies on the pharmacology of the perfused human fetal ductus arteriosus. *Am J Obstet Gynecol* **109:**937–942.

Melmon KL, Cline MJ, Hughes T and Nies AS (1968) Kinins: Possible mediators of neonatal circulatory changes in man. *J Clin Invest* **47:**1295–1302.

Mentzer RM, Ely SW, Lasley RD, Mainwaring RD, Wright EM and Berne RM (1985) Hormonal role of adenosine in maintaining patency of the ductus arteriosus in fetal lambs. *Ann Surg* **202:**223–230.

Millerd AL, Morales E, Leblanc NR and Cole WC (1993) Metabolic inhibition enhances Ca^{2+} activated K^+ current in smooth muscle cells of rabbit portal vein. *Am J Physiol* **265:**H2184–H2195.

Mlinar B and Enyeart JJ (1993) Block of current through T-type calcium channels by trivalent metal cations and nickel in neural rat and human cells. *J Physiol* **469:**639–652.

Moise KJ Jr, Huhta JC, Sharif DS, Ching-Nan O, Kirshon B, Wasserstrum N and Cano L (1988) Indomethacin in the treatment of premature labor: Effects on the ductus arteriosus. *N Engl J Med* **319:**327–331.

Moise KJ Jr, Ou CN, Kirshon B, Cano LE, Rognerud C and Carpenter RJ Jr (1990) Placental transfer of indomethacin in the human pregnancy. *Am J Obstet Gynecol* **162:**549–554.

Momma K and Takao A (1987) In vivo constriction of the ductus arteriosus by nonsteroidal anti-inflammatory drugs in near-term and preterm fetal rats. *Pediatr Res* **22:**567–572.

Momma K and Takao A (1989a) Increased constriction of the ductus arteriosus with combined administration of indomethacin and betamethasone in fetal rats. *Pediatr Res* **25:**69–75.

Momma K and Takao A (1989b) Right ventricular concentric hypertrophy and left ventricular dilatation by ductal constriction in fetal rats. *Circ Res* **64:**1137–1146.

Momma K, Hagiware H and Konishi T (1984) Constriction of fetal ductus arteriosus by non-steroidal anti-inflammatory drugs: Study of an additional 34 drugs. *Prostaglandins* **28:**527–536.

Momma K, Nishihara S and Ota Y (1981) Constriction of the fetal ductus arteriosus by glucocorticoid hormones. *Pediatr Res* **15:**19–21.

Momma K, Uemura S, Nishihara S and Ota Y (1980) Dilatation of the ductus arteriosus by prostaglandins and prostaglandin's precursors. *Pediatr Res* **14:**1074–1077.

Moncada S, Higgs A and Furchgott R (1997) XIV. International union of pharmacology: Nomenclature in nitric oxide research. *Pharmacol Rev* **49:**137–142.

Morita T, Perrella MA, Lee ME and Kourembanas S (1995) Smooth muscle cell-derived carbon monoxide is a regulator of vascular cGMP. *Proc Natl Acad Sci USA* **92:**1475–1479.

Nakanishi T, Gu H, Hagiwara N and Momma K (1993) Mechanisms of oxygen-induced contraction of ductus arteriosus isolated from the fetal rabbit. *Circ Res* **72:**1218–1228.

Needleman P, Homberg S and Mandelbaum B (1981) Ductus arteriosus closure may result from suppression of prostacyclin synthesis by an intrinsic hydroperoxy fatty acid. *Prostaglandins* **22:**675–682.

Nguyen MT, Camenisch T, Snouwaert JN, Hicks E, Coffman TM Anderson PAW, Malouf NN and Koller BH (1997) The prostaglandin receptor EP_4 triggers remodelling of the cardiovascular system at birth. *Nature* **390:**78–81.

Nishigaki N, Negishi M, Honda A, Sugimoto Y, Namba T, Narumiya S and Ichikawa A (1995) Identification of prostaglandin E receptor ' EP_2' cloned from mastocytoma cells as EP_4 subtype. *FEBS Lett* **364:**339–341.

Noel S and Cassin S (1976) Maturation of contractile response of ductus arteriosus to oxygen and drugs. *Am J Physiol* **231:**240–243.

Norton ME, Merrill J, Cooper BAB, Kuller JA and Clyman RI (1993) Neonatal complications after the administration of indomethacin for preterm labor. *N Engl J Med* **329:**1602–1607.

Okada M, Takeuchi J, Kimura H, Yoshijima K-I, Itoh K and Furuhashi T (1994) Histochemistry and immunohistochemistry of extracellular matrix of rat fetal ductus arteriosus during the indomethacin-induced constriction. *Exp Anim* **43:**551–558.

Pace-Asciak CR and Rangaraj G (1977) The 6-ketoprostaglandin $F_{1\alpha}$ pathway in the lamb ductus arteriosus. *Biochim Biophys Acta* **486:**583–585.

Pace-Asciak CR and Rangaraj G (1978) Prostaglandin biosynthesis and catabolism in the lamb ductus arteriosus aorta and pulmonary artery. *Biochim Biophys Acta* **529:**13–20.

Pace-Asciak CR and Rangaraj G (1983) Release of prostaglandins from intact lamb ductus arteriosus. *Can J Biochem Cell Biol* **61:**240–247.

Pierce KL, Gil DW, Woodward DF and Regan JW (1995) Cloning of human prostanoid receptors. *Trends Pharmacol Sci* **16:**253–256.

Pulkkinen MO, Momma K and Pulkkinen J (1986) Constriction of the fetal ductus arteriosus by progesterone. *Biol Neonate* **50:**270–273.

Rabinovitch M (1996) Cell-extracellular matrix interactions in the ductus arteriosus and perinatal pulmonary circulation. *Semin Perinatol* **20:**531–541.

Rasanen J and Jouppila P (1995) Fetal cardiac function and ductus arteriosus during indomethacin and sulindac therapy for threatened preterm labor: A randomized study. *Am J Obstet Gynecol* **173:**20–25.

Reeve HL, Tolarova S, Cornfield DN, Tristani-Firouzi M, Archer SL and Weir EK (1997) Developmental changes in K+ channel expression may determine the O_2 response of the ductus arteriosus (DA). *FASEB J* **11:**A420.

Riendeau D, Percival MD, Boyce S, Brideau C, Charleson S, Cromlish W, Ethier D, Evans J, Falgueyret JP, Ford-Hutchinson AW, Gordon R, Greig G, Gresser M, Guay J, Kargman S, Leger S, Mancini JA, O'Neill G, Ouellet M, Rodger IW, Therien M, Wang Z, Webb JK, Wong E, Xu L, Young RN, Zamboni R, Prasit P and Chan CC (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *Br J Pharmacol* **121:**105–117.

Roberts LI and Morrow JD (1997) The generation and actions of isoprostanes. *Biochim Biophys Acta* **1345:**121–135.

Roulet MJ and Coburn RF (1981) Oxygen-induced contraction in the guinea pig neonatal ductus arteriosus. *Circ Res* **49:**997–1002.

Sakurai H, Matsuoka R, Furutani Y, Imamura SI, Takao A and Momma K (1996) Expression of four myosin heavy chain genes in developing blood vessels and other smooth muscle organs in rabbits. *Eur J Cell Biol* **69:**166–172.

Sawdy R, Slater D, Jones G, Poston L and Bennet P (1997) Use of a cyclo-oxygenase type-2-selective non-steroidal anti-inflammatory agent to prevent preterm delivery. *Lancet* **350:**265–266.

Senior J, Marshall K, Sangha R and Clayton JK (1993) In-vitro characterization of prostanoid receptors on human myometrium at term pregnancy. *Br J Pharmacol* **108:**501–506.

Sharpe GL and Larsson KS (1975) Studies on closure of the ductus arteriosus X In vivo effect of prostaglandin. *Prostaglandins* **9:**703–719.

Sharpe GL, Thalme B and Larsson KS (1974) Studies on closure of the ductus arteriosus XI Ductal closure in utero by a prostaglandin synthase inhibitor. *Prostaglandins* **8:**363–368.

Shaul PW, Yuhanna IS, German Z, Chen Z, Steinhorn RH and Morin FC III (1997) Pulmonary endothelial NO synthase gene expression is decreased in fetal lambs with pulmonary hypertension. *Am J Physiol* **272:**L1005–L1012.

Sideris EB, Yokochi K, Coceani F and Olley PM (1985) Prostaglandins and fetal cardiac output distribution in the lamb. *Am J Physiol* **248:**H853–H858.

Silove ED, Coe JY, Shiu MF, Brunt JD, Page AJ, Singh SP and Mitchell MD (1981) Oral prostaglandin E_2 in ductus-dependent pulmonary circulation. *Circulation* **63:**682–688.

Silva DG and Ikeda M (1971) Ultrastructural and acetylcholinesterase studies on the innervation of the ductus arteriosus pulmonary trunk and aorta of the fetal lamb *J Ultrastruct Res* **34:**358–374.

Silver MM, Freedom RM, Silver MD and Olley PM (1981) The morphology of the human newborn ductus arteriosus. *Hum Pathol* **12:**1123–1136.

Simberg N (1983) The metabolism of prostaglandin E_2 in perinatal rabbit lung. *Prostaglandins* **26:**275–285.

Skidgell RA, Friedman WF and Printz MP (1984) Prostaglandin biosynthetic activities of isolated fetal lamb ductus arteriosus other blood vessels and lung tissue. *Pediatr Res* **18:**12–18.

Slomp J, Gittenberger de Groot AC, Glukhova MA, Van-Munsteren JC, Kockx MM, Schwartz SM and Koteliansky VE (1997) Differentiation dedifferentiation and

apoptosis of smooth muscle cells during the development of the human ductus arteriosus. *Arterioscler Thromb Vasc Biol* **17:**1003–1009.

Slomp J, Van-Munsteren JC, Poelmann RE, De-Reeder EG, Bogers AJ and Gittenberger De-Groot AC (1992) Formation of intimal cushions in the ductus arteriosus as a model for vascular intimal thickening an immunohistochemical study of changes in extracellular matrix components. *Atherosclerosis* **93:**25–39.

Smith GCS (1992) Detecting congenital heart disease prenatally. *Br Med J* **304:**578.

Smith GCS (1997) The effect of passive stretch on the response of the fetal rabbit ductus arteriosus to indomethacin noradrenaline and prostaglandin E₂. *Exp Physiol* **82:**537–546.

Smith GCS and McGrath JC (1988) Indomethacin but not oxygen tension affects the sensitivity of isolated neonatal rabbit ductus arteriosus but not aorta to noradrenaline. *Cardiovasc Res* **22:**910–915, 1988.

Smith GCS and McGrath JC (1991) Prostaglandin E₂ and fetal oxygen tension synergistically inhibit the response of the isolated fetal rabbit ductus arteriosus to noradrenaline. *J Cardiovasc Pharmacol* **17:**861–866.

Smith GCS and McGrath JC (1993) Characterisation of the effects of oxygen tension on the response of the fetal rabbit ductus arteriosus to vasodilators. *Cardiovasc Res* **27:**2205–2211.

Smith GCS and McGrath JC (1994) Interactions between indomethacin noradrenaline and vasodilators in the fetal rabbit ductus arteriosus. *Br J Pharmacol* **111:**1245–1251.

Smith GCS and McGrath JC (1995) Contractile effects of prostanoids on the fetal rabbit ductus arteriosus. *J Cardiovasc Pharmacol* **25:**113–118.

Smith GCS, Coleman RA and McGrath JC (1994) Characterization of dilator prostanoid receptors in the fetal rabbit ductus arteriosus. *J Pharmacol Exp Ther* **271:**390–396.

Starling MB and Elliot RB (1974) The effects of prostaglandins prostaglandin inhibitors and oxygen on the closure of the ductus arteriosus pulmonary arteries and umbilical vessels in vitro. *Prostaglandins* **8:**187–203.

Starling MB, Neutze JM, Eliott RL and Eliott RB (1976) Studies on the effects of prostaglandins E₁ E₂ A₁ A₂ on the lamb ductus arteriosus of swine in vivo using cineangiography. *Prostaglandins* **12:**355–367.

Tada T and Kishimoto H (1990) Ultrastructural and histological studies on closure of the mouse ductus arteriosus. *Acta Anat* **139:**326–334.

Tada T, Wakabayashi T, Nakao Y, Ueki R, Ogawa Y, Inagawa A, Shibata T and Kishimoto H (1985) Human ductus arteriosus: A histological study on the relation between ductal maturation and gestational age. *Acta Pathol Jpn* **25:**23–34.

Takizawa T, Arishima K, Yamamoto M, Somiya H and Shiota-K (1994a) Transplacentally-administered enalapril inhibits the spontaneous constriction of the ductus arteriosus in the newborn rat. *Congenital Anom* **34:**47–51.

Takizawa T, Oda T, Arishima K, Yamamoto M, Masaoka T, Somiya H, Akahori F and Shiota K (1994b) A calcium channel blocker verapamil inhibits the spontaneous closure of the ductus arteriosus in newborn rats. *J Toxicol Sci* **19:**171–174.

Takizawa T, Oda T, Arishima K, Yamamoto M, Somiya H, Eguchi Y and Shiota K (1994c) Inhibitory effect of enalapril on the constriction of the ductus arteriosus in newborn rats. *J Vet Med Sci* **56:**605–606.

Teitel DF, Iwamoto HS and Rudolph AM (1987) Effects of birth-related events on central blood flow patterns. *Pediatr Res* **22:**557–566.

Templeton AGB, McGrath JC and Whittle MJ (1991) The role of endogenous thromboxane in contractions to U46619 oxygen 5-HT and 5-CT in the human isolated umbilical artery. *Br J Pharmacol* **103:**1079–1084.

Terragno NA, Terragno A, McGiff JL and Rodriguez DJ (1977) Synthesis of prostaglandins by the ductus arteriosus of the bovine fetus. *Prostaglandins* **14:**721–727.

Thorburn GD (1992) The placenta PGE₂ and parturition, in *Advances in Perinatal Medicine* (Sakamoto S and Takeda Y, eds) pp 63–73, Elsevier, Amsterdam.

Tristani-Firouzi M, Reeve HL, Tolarova S, Weir EK and Archer SL (1996) Oxygen-induced constriction of rabbit ductus arteriosus occurs via inhibition of a 4-aminopyridine- voltage-sensitive potassium channel. *J Clin Invest* **98:**1959–1965.

Tsai MY and Brown DM (1987) Effect of dexamethasone on fetal lung 15-hyroxyprostaglandin dehydrogenase: Possible mechanism for the prevention of patent ductus arteriosus by maternal dexamethasone therapy. *Prostaglandins Leukotriene Med* **27:**237–245.

Tsai MY and Einzig S (1989) Prostaglandin catabolism in fetal and maternal tissues: A study of 15-hyroxyprostaglandin dehydrogenase and Delta 13 reductase with specific assay methods. *Prostaglandins Leukotriene Essent Fatty Acids* **38:**25–30.

Utz J and Ullrich V (1991) Carbon monoxide relaxes ileal smooth muscle through activation of guanylate cyclase. *Biochem Pharmacol* **41:**1195–1201.

Van Den Veyver IB and Moise KJ (1993) Prostaglandin synthetase inhibitors in pregnancy. *Obstet Gynecol Surv* **48:**493–502.

Walsh RS and Mentzer RM (1987) Role of cyclic nucleotides in relaxation of fetal lamb ductus arteriosus. *Surgery* **102:**313–318.

Walsh RS, Ely SW and Mentzer RM (1988) Response of lamb ductus arteriosus to nitroglycerin and nitroprusside. *J Surg Res* **44:**8–13.

Wang R and Wu L (1997) The chemical modification of K-Ca channels by carbon monoxide in vascular smooth muscle cells. *J Biol Chem* **272:**8222–8226.

Weiss H, Cooper B, Brook M, Schlueter M and Clyman RI (1995) Factors determining reopening of the ductus arteriosus after successful clinical closure with indomethacin. *J Pediatr* **127:**466–471.

Werkstrom V, Ny L, Persson K and Andersson KE (1997) Carbon monoxide-induced relaxation and distribution of haem oxygenase isoenzymes in the pig urethra and lower oesophagogastric junction. *Br J Pharmacol* **120:**312–318.

Wimsatt J, Nathanielsz PW and Sirois J (1993) Induction of prostaglandin endoperoxide synthase isoform-2 in ovine cotyledonary tissues during late gestation. *Endocrinology* **133:**1068–1073.

Yuan XJ, Tod ML, Rubin LJ and Blaustein MP (1995) Inhibition of cytochrome P₄₅₀ reduced voltage-gated K+ currents in pulmonary arterial myocytes. *Am J Physiol* **268:**C259–C270.

Zhu L, Dagher E, Johnson DJ, Bedell-Hogan D, Keeley FW, Kagan HM and Rabinovitch M (1993) A developmentally regulated program restricting insolubilization of elastin and formation of laminae in the fetal lamb ductus arteriosus. *Lab Invest* **68:**321–331.