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CHANGES IN VASCULAR SMOOTH MUSCLE REACTIVITY DURING DEVELOPMENT

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THE SCOPE OF THE REVIEW

Our understanding of the numerous factors that control vascular smooth muscle tone in the adult has advanced considerably in the last ten years. Salient points to emerge from the considerable amount of work done on the subject include a remarkable heterogeneity in vascular smooth muscle reactivity among different vascular beds and among different species and the variation in mechanisms and intensity of adrenergic control of different blood vessels (1). This explosion of knowledge about factors controlling blood vessel tone has only recently led investigators to explore in detail the way these mechanisms might change at different ages. A few exploratory studies have been done that point the way toward more intensive analysis.

This review has two purposes. The first is to summarize what is currently known about variations in factors that control vascular smooth muscle tone in the first few weeks of life. The second is to highlight methodological considerations that must be taken into account in order to optimize future studies in this area.

This review will focus on alterations in reactivity of vascular smooth muscle to neuronal influences and circulating substances during the first few weeks of life. The emphasis will be on in vitro studies because this approach allows one to study vascular smooth muscle reactivity without interference from reflex effects, differences in pharmacokinetics, etc. This does not imply that in vivo studies are not important or useful. Studies in vitro allow one to distinguish

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specific mechanisms that may be changing during growth. A basic knowledge of these mechanisms should make possible an improved design for in vivo studies in order to determine the physiological and pathological importance of changes in vascular smooth muscle reactivity. Indeed, a general overview of the in vivo approach is also included here.

This review will not deal with the dramatic changes in the cardiovascular system that occur at the time of birth and shortly thereafter. This aspect has recently been reviewed (2). Nor will we deal with studies of the factors that influence differentiation of smooth muscle or factors that determine the extent and pattern of innervation. Further information on these aspects can be found in several other reviews (3–5).

SPECIES VARIATION

Significant species differences in vascular smooth muscle reactivity have been defined in a single vascular bed (6, 7). However, more critical for studies of development are the differences in relative maturity at birth among various species. In the commonly used laboratory species this ranges from animals born in a highly developed state, such as guinea pigs and sheep, through pigs, dogs, and cats, to animals born in a highly immature state—rabbits and rats (8, 9). Thus, the age of the animal per se does not provide a good index of the stage of development when making comparisons between species. Throughout this review the age referred to is postnatal age unless otherwise specified.

Several researchers have claimed that pigs and dogs provide the closest model to the newborn human in terms of the stage of cardiovascular development (10–13). Indeed, a model of cerebral intraventricular hemorrhage, one of the most prevalent causes of morbidity and mortality in premature infants (14), has been developed in the newborn beagle (15).

THE GENERAL SCHEME OF ADRENERGIC NERVOUS SYSTEM ONTOGENY

From studies of the development of adrenergic nervous control of various organs a general principle can be derived: the function of the post-synaptic component can generally be demonstrated before the presynaptic elements are fully functional (16). Aspects of this process can be gleaned from studies on the vasculature and the general principle appears applicable to blood vessels. However, comprehensive studies are not yet available. Therefore, it is worthwhile to review the sequence of developmental events as they occur in a much more carefully studied tissue. The careful work of Slotkin's group, with its primary emphasis on the development of autonomic control of the heart,

provides an important model for studies of developmental changes in other organs.

Heart

At birth, activation of β-adrenergic receptors with isoproterenol produces both an increase in rate and force of contraction of the rat heart (17). However, responses to stimulation of sympathetic nerves, as tested using tyramine to release adrenergic transmitter, are small in the newborn and increase steadily over the first week of life (18). This increased responsiveness to adrenergic nerve activation can be correlated with development of the capacity of adrenergic nerves to accumulate norepinephrine by an active uptake mechanism (18). Cardiac norepinephrine content also increases steadily over the first two weeks of life (19).

It has been suggested that the key factor involved in the development of sympathetic neurotransmission is maturation of neurotransmitter synaptic vesicles, the organelle involved in storage and release of transmitter (20). This has been demonstrated both morphologically (20) and by measuring accumulation and content of norepinephrine.

While functional adrenergic nerves appear to be present in the heart of rats by one week of age, these nerves do not appear to be active either tonically or to play a role in reflex effects until a later stage of development. Treatment with the ganglionic blocker chlorisondamine does not affect heart rate in animals one week of age or younger, although heart rate is lowered by chlorisondamine in rats eleven days of age (17). In adult rats, administration of the vasodilator hydralazine causes a depletion of cardiac norepinephrine levels by reflex activation of sympathetic nerves. This effect of hydralazine cannot be seen in rats until fourteen days of age (19), nor does hydralazine evoke reflex tachycardia before fourteen days of age (21). Thus, immaturity of reflex activation must reside either in the afferent or sensory limb of this reflex or within the central nervous system itself.

The ability of the enzyme ornithine decarboxylase to respond to β-adrenergic activation of the heart has been used to trace the development of cardiac adrenergic control systems. The enzyme ornithine decarboxylase has a rather short turnover time, so its activity can alter rapidly (22, 23). Since this enzyme is sensitive to a number of specific hormones and neurotransmitters, it can provide a very sensitive index of responsiveness of an organ to direct or indirect stimuli. Thus, activity of ornithine decarboxylase has been used as an index of functional development of the peripheral nervous system (24).

In the first five days of life, administration of isoproterenol to rats increases ornithine decarboxylase activity, but administration of nicotine, which acts through adrenergic nerves, does not affect ornithine decarboxylase activity

until animals are older than six days of age (25). In addition, insulin-induced hypoglycemia, which stimulates ornithine decarboxylase activity in the adult rat heart by reflex sympathetic activation, has no effect in the neonatal rat heart until eight days of age (26). This confirms the immaturity of sympathetic control of the heart in rats younger than one week.

Adrenal Medulla

Maintenance of cardiovascular function in the newborn may be more dependent on activity of the adrenal medulla than is true of the adult. In adrenalectomized newborn puppies, ability to maintain responsiveness of the heart to repeated and prolonged sympathetic stimulation is reduced in comparison to sham-operated dogs (27).

In addition, mechanisms of responsiveness of the adrenal medulla in immature animals appear to be unique. In the immature sheep and calf, hypoxia produces an adrenal medullary discharge, primarily of norepinephrine, which is not dependent on an intact splanchnic innervation (28). In the sheep, this unique response disappears before birth, while in the less mature newborn calf it is present until 24 hours after birth.

The time course of development of adrenal medullary control mechanisms has been explored most intensively in the rat (20). Stimulation of the splanchnic nerve of the newborn rat does not produce a response in the adrenal. Furthermore, insulin-induced hypoglycemia, which in the adult produces sympathetic activity, does not cause release of adrenal medullary catecholamines from the newborn rat. However, direct activation of the adrenal medulla by nicotine, which activates cholinergic receptors in the organ itself, does cause release of catecholamines in the neonatal rat. Thus, the limiting factor in the development of adrenergic control of this organ is not development of reactivity of the organ itself but rather development of splanchnic nerve function.

Vascular Smooth Muscle

Much less work has been done on the development of adrenergic control of vascular smooth muscle than of the heart. The situation is further complicated by the marked heterogeneity among different vascular beds of mechanisms controlling blood vessel tone. Those studies that have been done suggest that the same general principle elaborated above applies to vascular smooth muscle: the capability of vascular smooth muscle to respond to circulating substances develops before functional adrenergic nerves are present (29). Thus, the fetal lamb carotid artery contracts in vitro to norepinephrine before responses to adrenergic nerve stimulation can be detected. By a gestational age of 115–130 days responses to adrenergic nerve stimulation can be detected, but their magnitude continues to increase until 140–150 days of gestational age. Thus, in

the lamb, which is born in a highly developed state, development of blood vessel reactivity predates establishment of functional adrenergic nerve connections.

BLOOD VESSELS

Adrenergic Innervation

There are a number of different approaches to assessing the density or functional capacity of sympathetic innervation. These include histological assessment using catecholamine fluorescence (30, 31); measurement of cocaine-sensitive accumulation of ^{3}H-norepinephrine (32) or an unmetabolized analog, ^{3}H-metaraminol (33, 34); and measurement of endogenous norepinephrine content and assessment of activities of catechol-o-methyl transferase and monoamine oxidase, enzymes that metabolize norepinephrine. The unique localization of adrenergic nerves to the adventitia and advential-medial border of blood vessels makes it difficult to determine what is the most appropriate index for evaluating the density of adrenergic innervation. Since the adrenergic nerve plexus approaches a two-dimensional form, comparisons based on tissue weight may be misleading if the smooth muscle cell layer differs greatly in thickness, as may be true at different stages of growth. The best solution to this problem is to include more than one index of nerve density. A histochemical approach using quantitative image analysis has proved particularly productive (35).

In the rabbit at one week before birth adrenergic nerves appear straight, smooth, and faintly fluorescent in the blood vessel wall, with few varicose swellings (35). By one day before birth, the number of nerves has increased markedly and more varicosities can be seen. A period of rapid nerve growth in the large arteries then occurs until one day after birth, with large increases in number of varicosities in the carotid, renal, and femoral arteries.

The time of development of adrenergic innervation varies considerably among blood vessels. The femoral artery has almost reached the adult level of varicosity number by one day after birth. Smaller arteries, such as the mesenteric artery and the basilar artery, have very sparse nerve plexuses at one day after birth, and adrenergic nerves in these vessels show a rapid growth period between one day after birth and six weeks of age.

Similar conclusions about the developmental pattern of vascular adrenergic innervation can be drawn from other studies. In the rat portal vein, development of the two layers of muscle characteristic of this tissue can be observed at four days after birth. At this point, adrenergic nerves are restricted to the outer layer of the vessel wall. For the next two weeks adrenergic nerve fibers penetrate through the outer longitudinal muscle layer to establish a two-dimensional plexus between the two muscle layers (36).

Studies of three rat arteries, the tail, superficial epigastric, and saphenous, show patterns of nerve development similar to what was found in the rabbit (37). A comparison of rabbit and guinea pig renal arteries shows a decline in adrenergic nerve density in the guinea pig renal artery from birth to four weeks, while rabbit renal arteries continue to gain in adrenergic nerve density for up to six months (35, 38).

These differences in time course of nerve development have been primarily studied in large arteries and it is difficult to extrapolate from this to true resistance vessels. Despite this limitation, such studies make it clear that at least three phases of nerve growth can be distinguished (35). The first phase involves outgrowth of new axons, which in the rabbit may take place either before or after birth. In the second phase of growth, regions of intense fluorescence appear along the nerve. This presumably corresponds to development of transmitter synthesis and storage capacity. This is followed by a period of rapid growth and differentiation of adrenergic nerve varicosities. In large arteries of the rabbit, this takes place shortly after birth, but in many blood vessels this period may extend for several weeks after birth.

In Vitro Studies

MECHANICAL PROPERTIES Changes that occur in the structure and mechanics of arteries during development have been reviewed previously (39). In some specialized vessels, for example the pulmonary artery, the time of birth produces profound specific changes in structure and function, including a slight thickening and a decline in mechanical stiffness. In most vessels, however, development is associated with a marked thickening of the vessel wall and progressively increasing mechanical stiffness.

The structure of the rat aorta has been carefully studied during development (40). During the first ten to fifteen weeks of life, the rat aorta increases in length to correspond with a rapid increase in total body weight and crown rump length. The bulk of cell division in the rat aorta as measured by autoradiographic incorporation of ^{3}H-thymidine occurs during the first weeks of life in the thoracic segment and during the first eighteen days in the abdominal segment. Total DNA and RNA content of the developing rat aorta increases markedly in the first five weeks of life, although when expressed as a function of dry weight there is a marked decrease in both DNA and RNA content (41). This corresponds to a decrease in number of cells per unit dry weight during growth; thus, there is a decrease in cellularity of the entire aorta during the first month of life. Both collagen and elastin, as total amounts and when expressed per unit dry weight, show increases during development in the rat aorta (41). The amount of collagen continues to increase up to fifteen weeks of age, while elastin content per unit dry weight reaches a peak by eighteen days of life and then shows a progressive decline to twenty-two weeks of age.

Similar results were found in a study of the dog carotid artery (42). Collagen content as a function of wet weight increased from two to twenty-eight weeks of age, while elastin content increased slightly from two to seven weeks and then declined to reach a constant level by twenty-eight weeks. This corresponds to an increase in collagen-to-elastin ratio between seven and twenty-eight weeks of life.

Analysis of extracellular and intracellular water and electrolyte contents shows that in the canine carotid artery there is a decrease in total water content, primarily due to a decrease in extracellular water space as measured by distribution of ^{60}CO-EDTA (42–44). Intracellular water space does not change during development. Total electrolyte content of the canine aorta and carotid artery, including sodium, potassium, chloride, magnesium, and calcium, also declines in the first few weeks of life (43, 44). In addition, with increasing age, efflux of $^{42}K^+$ slows in both the canine aorta and carotid artery.

The effect of these developmental changes in structure and connective tissue content on mechanical properties of large blood vessels has also been studied (42, 43). Because of the rapid alteration in tissue diameter during growth, in order to compare mechanical properties of vessels from animals of different ages diameter at each pressure is normalized by dividing by the diameter at zero pressure. Passive tangential stress-strain curves are shifted progressively with increasing age so that for a given normalized external diameter the passive tangential stress is increased in older animals. This has been shown for canine iliac, renal, carotid, and mesenteric arteries, and aorta. These changes have been interpreted as indicating an increase in arterial wall stiffness with age, corresponding to a decrease in cellularity and increase in connective tissue content.

SMOOTH MUSCLE RESPONSIVENESS Several different approaches can be used for investigating vascular smooth muscle responsiveness in the tissue bath: vessel segments can be perfused or isometric or isotonic contractions can be measured using ring segments or strips (45). For determination of receptor characteristics in vascular smooth muscle, isometric recording is widely used. Effects of drugs or transmitter substances added to the bath can be determined, or nerves in the vessel wall can be stimulated using transmural electric pulses (46).

For studies of isometric contraction, the resting tension placed on the vessel is critical, since the active force developed is dependent on the passive force applied to the tissue (39). In order to maintain vessels of different sizes or mechanical properties at the same point on the length tension curve, these should all be studied at the optimum resting tension. This is defined as the resting tension at which active contractile responses are maximized.

Once the appropriate resting tension for a given vessel is determined, one

must then decide how to control for changes in structure, orientation of smooth muscle cells, etc. In this regard, a measure of contractile ability that is not specific for a single receptor mechanism has been useful. For example, activation with a high concentration of potassium has been used as a non-specific contractile stimulus. If one wishes to examine changes in responsiveness to activation of a specific receptor type, for example the β-adrenergic receptor, then it is important to select experimental conditions that involve activation of that receptor alone, without interference from other mechanisms (47). Methods for analysis of dose-response data and for determination of receptor characteristics using smooth muscle responses have been reviewed (48).

In the last ten years powerful radioligand binding methods have been developed that make it possible to look at characteristics of receptors themselves (49). While these techniques are extremely useful, one should remember that they give little indication of alterations in the multiple processes involved in receptor-contraction coupling.

β-Adrenergic stimulation One of the best defined age-dependent alterations in vascular smooth muscle reactivity is the change in magnitude of the β-adrenergic response (50). In both rabbit and rat aorta, as well as several other arteries, β-adrenergic relaxation declines with advancing age. For example, in rabbit aorta isoproterenol-induced relaxation declines from 56–70 days of age, so that in aortae from animals three to five years of age there is no relaxation response to isoproterenol (51, 52). This age-related change has been shown to be altered by variation in diet (53). Aortae from rats fed a restricted diet (60% of amount consumed by controls) showed a greater decline in the magnitude of β-adrenergic relaxation than aortae from control rats.

Since the magnitude of drug-induced relaxation depends on the level of initial smooth muscle tone, it is important in such studies to use equivalent levels of contraction in vessels from animals of different ages (54). One way to demonstrate specificity of alterations in relaxation magnitude is to use several different drugs that act by different mechanisms to produce relaxation. For example, if a specific change in β-adrenergic stimulation occurs, then relaxation responses to nitroglycerin, nitrite, or papaverine should not change (51, 54). Studies of the rat aorta showed that when the level of smooth muscle tone was equated on the basis of percent maximal tension, the magnitude of nitroglycerin-induced relaxation was not constant. Only when a similar level of grams force was maintained did the nitroglycerin-induced relaxation remain constant in aortic strips from animals of different ages.

Closer examination of the first few weeks of life shows that β-adrenergic mechanisms are not fully developed in the newborn. In aortic strips from rabbits 1–2 days old, only small relaxations to isoproterenol are seen, and these reach a maximal level by one month of age. In contrast, the magnitude of

relaxation to sodium nitrite or papaverine stays constant at all ages. Relaxation responses to adenosine also show an age-related decline, so that aortae from rabbits 360 days old show very little relaxation response to this substance (55).

α-Adrenergic stimulation The magnitude of β-adrenergic relaxation changes with age, and many substances, such as norepinephrine, that stimulate α-adrenergic receptors also activate β-adrenergic receptors. Therefore, in many studies where β-adrenergic responses are not blocked it is difficult to draw conclusions about changes in α-adrenergic mechanisms per se. For example, it has been shown that the β-adrenergic antagonist propranolol potentiates responses to norepinephrine in aortae from immature (2–12 days) rabbits, but to a much lesser extent in aortae from rabbits 90 days of age (55). Thus, the much greater β-adrenergic relaxation present in aortae from young animals has a greater impact on responses to norepinephrine than in aortae from older animals, where there is much less β-adrenergic response.

A more fundamental problem in the interpretation of many studies is that no control of the profound changes in blood vessel size and mechanics is included. Since in some cases data are not normalized to the maximum contractile response of the tissue, it is difficult to decide whether increased responses to α-adrenergic stimulation are due to growth of the vessel or to a relative increase in development of α-adrenergic responsiveness.

In most studies, however, there appears to be an increase in maximal responses to norepinephrine in the first few weeks of life when these are corrected for the contractile ability of the tissue. In canine iliac, carotid, renal, and mesenteric arteries, vessels from animals younger than two weeks of age showed smaller responses to α-adrenergic stimulation relative to responses to KCl compared to adults (43). However, in the canine aorta one study showed no change in α-adrenergic responsiveness with age (56). In fetal sheep, the maximum response to norepinephrine as a percent of maximum response to serotonin increases when animals 115–130 days of gestation are compared to fetuses at 140–150 days (29).

When sensitivity to α-adrenergic stimulation is examined, there is much more disagreement among studies. In several studies no change in the norepinephrine EC_{50} (concentration for 50% of the maximal response) has been noted [sheep fetus (29), sheep ear artery (57), guinea pig and rabbit renal arteries (38), rat portal vein (58)]. However, a decrease in EC_{50} for both norepinephrine and phenylephrine has been found in the canine aorta and for norepinephrine in the sheep carotid (56, 59). In guinea pig and rabbit renal arteries, there was no difference in norepinephrine EC_{50} when newborn and adult animals were compared. However, the slope of the concentration-response curve was different. At low concentrations the newborn guinea pig renal artery was more sensitive to norepinephrine than the adult, while the adult rabbit renal

artery was more sensitive than the newborn at low norepinephrine concentrations (38). The phentolamine pA_2, an estimate of the antagonist dissociation constant, did not change for the sheep ear artery from animals of five age groups ranging from 110 day-old fetuses to adults (57).

Examination of the rabbit mesenteric and intestinal microvasculature showed no change in the ability of norepinephrine to produce arteriolar constriction from day 23 of gestation to the adult (60). However, the duration of the response was longer in older fetuses and the adult.

Responses to adrenergic nerve stimulation show clear-cut developmental changes that correspond to the growth of adrenergic nerves during this time period. In the sheep ear artery and carotid artery, responses to nerve stimulation show a progressive increase from 110 days of gestation to the adult (29, 57). Newborn rabbit renal arteries also have a relatively smaller response to nerve stimulation than vessels from the adult (38). Responses to nerve stimulation have also been studied using tyramine, which releases transmitter from adrenergic nerves, showing a similar pattern of development of adrenergic responsiveness (56, 58).

Significant species differences have been highlighted in studies of the portal vein (8). The newborn guinea pig portal vein responds as well as the adult's to nerve stimulation, while the newborn rat portal vein does not contract to nerve stimulation. Intermediate are portal veins of rabbits and cats, which do have a small response to nerve stimulation that becomes much larger in the adult.

Other vasoactive substances There is considerable variability in the developmental profile of other types of vasoactivity. For example, the spontaneous activity seen in the portal vein is present at birth in the guinea pig but absent in the newborn rabbit and cat (8). In the rat portal vein, spontaneous activity does not develop until 15–20 days of age (58).

Responses to serotonin show a decrease in EC_{50} during development in the sheep carotid artery (29, 59), but no change in the sheep ear artery (57). The maximal response to serotonin declined in the sheep carotid artery from 250% of the norepinephrine response at 53–90 days gestation to 112% at 140–150 days (57). In the rabbit aorta, the maximal contractile response to serotonin declined relative to the norepinephrine response from 5 to 360 days of age (55).

The EC_{50} for angiotensin was shown to be higher in the newborn sheep carotid artery than in the adult (59), while responses to vasopressin did not change during development in the sheep ear artery (57). The EC_{50} for acetylcholine did not change with age in the rat portal vein (58).

In Vivo Studies

In some species, depending on the state of maturity at birth, reflex responses of the vasculature mediated by adrenergic nerves may be incompletely developed

in the newborn. In other species, however, responses may be indistinguishable from the adult. In pigs, fully integrated vascular responses to reflex activation of the adrenergic nervous system are not fully developed until two weeks of age (61, 62).

In the mature-at-birth sheep, the magnitude of neurohumoral control was assessed by the change in blood pressure produced by α-adrenergic blockade with phenoxybenzamine or ganglionic blockade with trimethaphan. Responses to blockade of neurohumoral control were greater in the term fetus than in the neonate and declined with age to the adult (63), suggesting more intense sympathetic control of blood pressure in the term fetus and newborn than in the adult.

In the dog, on the other hand, some studies seem to demonstrate that vascular responses to adrenergic nerve stimulation are not fully developed in the newborn. Sympathetic nerve stimulation of the isolated perfused hindquarters of the newborn dog produced a vasodilator response that was blocked by atropine. Not until two weeks of age was a vasoconstrictor response to nerve stimulation seen (64). Bilateral carotid occlusion produced an increase in blood pressure in newborn dogs, although the magnitude of this increase was smaller than that in dogs ten days old or in adults (65). In contrast, though, the indirectly acting adrenergic agonist tyramine produced similar pressor effects in newborn and adult dogs (66).

Arterial blood pressure in the newborn is low and increases progressively to the adult level in a number of species that have been studied (65, 67, 68). This makes it difficult to decide how to compare blood pressure responsiveness in animals of different ages, since the absolute amount of change in blood pressure produced by a given stimulus would be expected to alter just on the basis of differences in resting blood pressure. To circumvent this problem, some authors have expressed changes as percent of resting blood pressure.

Despite these difficulties in interpretation of in vivo data, studies of responses to adrenergic drugs confirm conclusions reached from in vitro studies. In the newborn pig, higher doses of isoproterenol are necessary to produce a fall in systemic blood pressure, and for a given dose of isoproterneol there was a smaller decrease in resistance as calculated from femoral and carotid arterial flows (69). Thus, in pigs β-adrenergic responsiveness of vascular smooth muscle is apparently still developing during the second postnatal week. Intravenous injections of isoproterenol also had much smaller effects on blood pressure in the term fetus of the sheep than in the adult (63).

Confounding effects of differences in drug disposition and metabolism are illustrated by the study of Boatman et al (64). Injections of epinephrine and norepinephrine produced similar increases in pressure in the isolated perfused hindquarters of the dog at all ages studied. However, the effective concentration of the drug was less in the adult due to its much larger size. These authors,

therefore, concluded that newborn dogs were much less sensitive to adrenergic drugs than the adult. In addition, they noted that the duration of the response in the newborn was much longer than in the adult and suggested that there are differences in metabolism of adrenergic agonists perhaps related to immaturity of adrenergic nerves. In another study of the dog, there was no change in blood pressure response to norepinephrine, although, again, the response duration was longer in the newborn than in the adult (66).

In newborn pigs, higher doses of norepinephrine were necessary to produce a significant change in blood pressure compared to adults (69). In addition, the absolute magnitude of blood pressure change was greater in the adult, although the significance of this observation is difficult to interpret.

Studies of blood pressure in the sheep show no significant alteration in maximal responses to norepinephrine with age (63). However, comparison of dose response curves to norepinephrine indicate that the term fetus showed considerably less sensitivity to norepinephrine than the neonate.

Examination of blood pressure responses to angiotensin in the lamb showed no alterations in the newborn lamb from birth to 60 days of age, even though baseline blood pressure rose progressively (70). In these animals, maintained on a carefully monitored sodium intake, the rise in arterial pressure with age could not be accounted for by a change in vascular sensitivity to angiotensin.

To this date, most in vivo studies have focused on changes in systemic blood pressure. However, as shown by morphological and in vitro studies, there are significant differences among vascular beds in the rat of maturation of adrenergic mechanisms. Therefore, it will be important in future studies to carefully analyze regional variations in vascular adrenergic mechanisms.

CLINICAL CORRELATES

From the clinician's standpoint, a major impetus to understanding the way in which the vascular system develops is the important role that this system plays in the adaptation of the newborn to the extrauterine environment. With advances taking place in the management of the pulmonary components of hyaline membrane disease, the major clinical challenges facing us in the care of premature and full-term infants are vascular disorders of adaptation. Three disorders in which the vasculature appears to play a major role are persistent fetal circulation, intraventricular hemorrhage, and necrotizing enterocolitis.

Persistent Fetal Circulation

The transition occurring at the moment of birth is the single most dramatic physiologic event in the life of a human (71). In those few seconds the pattern of circulation must change from one based on placental transfer of oxygen to one allowing pulmonary exchange of gases. These changes are primarily vascular

in origin, resulting in vasodilation of the pulmonary vessels and constriction of the umbilical arteries. They are uniquely characteristic of the infant born after 37 weeks' gestation. At younger ages, or in cases where the birth process is complicated by severe asphyxia, these vascular transitions may not occur or may be altered. This abnormal circulatory state has been termed persistent fetal circulation and is manifest primarily as pulmonary hypertension and failure of the ductus arteriosus to close (72).

During the normal birth process, at the instant of the first breath the resistance in the pulmonary vasculature drops dramatically and the ductus arteriosus closes. The mediators of this process have not been defined, although the compounds most commonly implicated are the prostaglandins. Evidence for a partial role of prostaglandins in "first-breath" vasodilation has been described (73). However, the failure of indomethacin to completely inhibit the "first-breath" effect suggests that other mediators may also be involved.

Following an acute episode of asphyxia, the normal pulmonary vasodilation associated with the "first breath" fails (72). While factors such as hypoxia and systemic acidemia have been implicated as contributing factors, the cellular or chemical mediators that cause pulmonary vasoconstriction to persist have not been identified. In the preterm infant, unknown chemical mediators associated with prematurity and hyaline membrane disease may contribute to decreased pulmonary blood flow.

The various approaches to treatment of this disorder further underline our lack of basic information as to etiology. Tolazoline is one frequently recommended drug for persistent fetal circulation (74). This drug has histaminergic, α-adrenergic, and cholinergic properties (75). In some species, the vasodilator properties of tolazoline have been attributed to stimulation of histamine type 1 and 2 receptors (76), while in other species it has been suggested that the efficacy of this drug is related to α-adrenergic antagonism (77). Without some basic understanding of the mechanisms involved in the human neonatal response to a drug such as tolazoline, it will be impossible to predict or design drug regimens that would be most efficacious in treating this disorder.

Intraventricular Hemorrhage

Until the 36th week of gestation in the human infant, there exists an area in the brain at the level of the caudate nucleus that is referred to as the subependymal germinal matrix layer (78). This layer gives rise to cells that migrate out and further develop the neuronal elements of the central nervous system. In its role as a center of growth, this area is richly supplied with a network of capillary blood vessels. Thus, the premature infant has a unique vascular structure that is absent at full-term normal birth. On the basis of cumulative evidence (79–81), it appears that this vascular bed is predisposed to hemorrhage into the ventricular system when subjected to asphyxial conditions followed by surges of

cerebral blood flow. In a prospective study (82), evidence of hemorrhage in the subependymal area was present in 78% of all premature infants admitted into an intensive-care nursery. This process of acute hemorrhage is associated with a high incidence of nonobstructive hydrocephalus as well as focal and global neurologic deficits.

Because of the severity of this problem, much effort has been directed toward an understanding of the etiology of these acute hemorrhages. In an animal model (83), intraventricular hemorrhage can be produced by asphyxia, hypertension (secondary to catecholamine infusion), and infusions of substances causing an increase in plasma volume and/or osmolality. This has led investigators (84) to speculate that autoregulation may not be intact in the normal premature newborn. Based on work using xenon washout in premature infants, it has been suggested that flow to the premature infant's brain is pressure passive, with only very limited autoregulatory capabilities. This evidence has led Volpe (85) to hypothesize that the richly vascularized subependymal germinal matrix layer has a poorly developed supportive structure and is injured by hypoxia and hypoperfusion. Following this injury, any pressure-passive surges in blood flow to the area result in rupture of the capillary and precapillary vessels.

It is apparent that if cerebral blood flow is indeed pressure passive in the neonate, then events that alter the systematic arterial blood pressure may have a dramatic effect on the central nervous system. Thus, infants with cardiovascular compromise on the basis of hyaline membrane disease and secondary pulmonary vasoconstriction or altered blood flow pathways (such as through a ductus arteriosus) may also have associated intraventricular hemorrhage.

In addition, the implications for therapeutic intervention are great. The use of vasoactive substances that have no effect on the cerebral blood vessels but cause increases in peripheral resistance and pressure may be deleterious to the newborn. Current efforts to minimize the incidence of intraventricular hemorrhage have all centered around elimination of high-risk situations (86). These attempts, while perhaps able to decrease the incidence of this problem, fail to address the underlying etiology of either a lack of or a failure of the cerebral autoregulatory mechanisms. Only by understanding the mechanisms associated with control of cerebral blood flow in the neonate can reasonable therapeutic choices be made in emergency situations requiring aggressive support of vital signs.

Necrotizing Enterocolitis

A third clinical entity of major concern in neonatal intensive care units is necrotizing enterocolitis. While the exact etiologic role of vascular smooth muscle in this disorder is not clear, epidemiologic evidence points to cardiovascular disorders as a major risk factor (87). This is primarily a disease of

premature infants; the majority weigh less than 2.5. kg at birth and have a gestational age of less than 38 weeks (88). For all neonatal intensive care units across the United States, an average of about 3000 deaths per year may occur as a results of this disorder (89).

Cardiovascular risk factors associated with the development of necrotizing enterocolitis include cyanotic congenital heart disease, patent ductus arteriosus, umbilical artery catheterization, and exchange transfusion (87). In addition, it has been recognized that unique cardiovascular reflexes present in the perinatal period may contribute to hypoperfusion states for the mesenteric circulation. It has been noted that asphyxiated newborn infants develop bradycardia and an increase in lactate concentration in the blood (90). These responses are similar to the so-called diving reflex, a phenomenon observed in the diving seal where the heart rate drops and the renal, mesenteric, and peripheral circulations are vasoconstricted to maintain maximal blood flow to the central nervous system (91). This similarity has led to speculation that the normal diving reflex may lead to mesenteric ischemia, particularly in the preterm infant who is asphyxiated.

In infants with cyanotic congential heart disease or vascular shunts causing rapid runoff of arterial circulation (such as patent ductus arteriosus), this mesenteric ischemia may be further exaggerated. In a similar manner, emboli associated with procedures such as umbilical artery catheterization may also cause mesenteric ischemia. If one accepts mesenteric ischemia as a primary event in the development of necrotizing enterocolitis, it follows that ischemic breakdown of the mucosal defense barriers may contribute to invasion of bacteria and further breakdown of the gut endothelium.

From an empirical standpoint, the prevention of necrotizing enterocolitis should involve prevention of asphyxial states and, thus, the avoidance of the diving-seal phenomenon. In addition, care should be taken to prevent other vascular embolic events, such as those associated with use of indwelling catheters. This provides us with a minimal clinical base from which to operate, however. Without an understanding of the underlying mechanisms involved in local regulation of the vascular responses of the mesenteric bed, it is impossible to rationally treat infants who have been exposed to asphyxial episodes. Basic research should be directed at an understanding of the effects of endogenous and exogenous vasoactive compounds in order to optimize and restore blood flow to critical areas in the asphyxiated neonate.

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Literature Cited

1. Bevan, J. A., Bevan, R. D., Duckles, S. P. 1980. Adrenergic regulation of vascular smooth muscle. In *Handbook of Physiology, Section 2, Cardiovascular System: Vascular Smooth Muscle*, ed. D. F. Bohr, A. P. Somlyo, H. V. Sparks, 2:515-66. Bethesda, Md: Am. Physiol. Soc. 686 pp.
2. Heymann, M. A., Iwamoto, H. S., Rudolph, A. M. 1981. Factors affecting changes in the neonatal systemic circulation. *Ann. Rev. Physiol.* 43:371–83
3. Hendry, I. A. 1976. Control in the development of the vertebrate sympathetic nervous system. In *Reviews of Neuroscience*, ed. S. Ehrenpreis, I. J. Kopin, 2:149–94. New York: Raven. 270 pp.
4. Black, I. B. 1978. Regulation of autonomic development. *Ann. Rev. Neurosci.* 1:183–214
5. Burnstock, G. 1981. Development of smooth muscle and its innervation. In *Smooth Muscle*, ed. E. Bulbring, A. F. Brading, A. W. Jones, T. Tomita, pp. 431–57. Austin: Univ. Tex. Press. 563 pp.
6. Ducklcs, S. P., Lcc, T. J.-F., Bevan, J. A. 1977. Cerebral arterial responses to nerve stimulation in vitro. Species variation in the constrictor and dilator components. In *Neurogenic Control of the Brain Circulation*, ed. C. Owman, L. Edvinsson, pp. 133–142. New York: Pergamon. 510 pp.
7. Duckles, S. P. 1979. Neurogenic dilator and constrictor responses of pial arteries in vitro. Differences between dogs and sheep. *Circ. Res.* 44:482–90
8. Stage, D., Ljung, B. 1978. Neuroeffector maturity of portal veins from newborn rats, rabbits, cats and guinea pigs. *Acta Physiol. Scand.* 102:218–23
9. Buckley, N. M., Gootman, P. M., Yellin, E. L., Brazeau, P. 1979. Age-related cardiovascular effects of catecholamines in anesthetized piglets. *Circ. Res.* 45: 282–92
10. Haworth, S. G., Hislop, A. A. 1981. Adaptation of the pulmonary circulation to extra-uterine life in the pig and its relevance to the human infant. *Cardiovas. Res.* 15:108–19
11. Ment, L. R., Duncan, C. C., Stewart, W. B. 1983. Local cerebral blood flow and metabolism alterations in the newborn beagle puppy model of intraventricular hemorrhage, In *Concepts in Pediatric Neurosurgery*, ed. A. J. Raimondi, 3:108–24. Basel: Karger. 224 pp.
12. Book, S. A., Bustad, L. K. 1974. The fetal and neonatal pig in biomedical research. *J. Anim. Sci.* 38:997–1002
13. Glauser, E. M. 1966. Advantage of piglets as experimental animals in pediatric research. *Exp. Med. Surg.* 24:181–90
14. Volpe, J. J. 1981. Current concepts in neonatal medicine. Neonatal intraventricular hemorrhage. *N. Engl. J. Med.* 304:886–91
15. Goddard, J., Lewis, R. M., Armstrong, D. L., Zeller, R. S. 1980. Moderate, rapidly induced hypertension as a cause of intraventricular hemorrhage in the newborn beagle model. *J. Pediatrics* 96:1057–60
16. Pappano, A. J. 1977. Ontogenetic development of autonomic neuroeffector transmission and transmitter reactivity in embryonic and fetal hearts. *Pharmacol. Rev.* 29:3–33
17. Seidler, F. J., Slotkin, T. A. 1979. Presynaptic and postsynaptic contributions to ontogeny of sympathetic control of heart rate in the preweanling rat. *Brit. J. Pharmacol.* 65:431–34
18. Bareis, D. L., Slotkin, T. A. 1980. Maturation of sympathetic neurotransmission in the rat heart. I. Ontogeny of the synaptic vesicle uptake mechanism and correlation with development of synaptic function. Effects of neonatal methadone administration on development of synaptic vesicles. *J. Pharmacol. Exp. Ther.* 212:120–25
19. Seidler, F. J., Slotkin, T. A. 1981. Development of central control of norepinephrine turnover and release in the rat heart: Responses to tyramine, 2-deoxyglucose and hydralazine. *Neuroscience* 6:2081–86
20. Slotkin, T. A., Smith, P. G., Lau, C., Bareis, D. L. 1980. Functional aspects of development of catecholamine biosynthesis and release in the sympathetic nervous system. In *Biogenic Amines in Development*, ed. H. Parvez, S. Parvez, pp. 29–48. New York: Elsevier/North Holland. 735 pp.
21. Bartolome, J., Mills, E., Lau, C. Slotkin, T. A. 1980. Maturation of sympathetic neurotransmission in the rat heart. V. Development of baroreceptor control of sympathetic tone. *J. Pharmacol. Exp. Ther.* 215:596–600
22. Russell, D. H., Snyder, S. H. 1968. Amine synthesis in rapidly growing tissues: Ornithine decarboxylase activity in regenerating rat liver, chick embryo and

various tumors. *Proc. Natl. Acad. Sci. USA* 60:1420–27
23. Russell, D. H., Snyder, S. H. 1969. Amine synthesis in regenerating rat liver: Extremely rapid turnover of ornithine decarboxylase. *Mol. Pharmacol.* 5:253–62
24. Slotkin, T. A. 1979. Ornithine decarboxylase as a tool in development neurobiology. *Life Sci.* 24:1623–30
25. Bartolome, J., Lau, C., Slotkin, T. A. 1977. Ornithine decarboxylase in developing rat heart and brain: Role of sympathetic development for responses to autonomic stimulants and the effects of reserpine on maturation. *J. Pharmacol. Exp. Ther.* 202:510–18
26. Bareis, D. L., Slotkin, T. A. 1978. Responses of heart ornithine decarboxylase and adrenal catecholamines to methadone and sympathetic stimulation in developing and adult rats. *J. Pharmacol. Exp. Ther.* 205:164–74
27. Erath, H. G., Boerth, R. C., Graham, T. P. Jr. 1982. Functional significance of reduced cardiac sympathetic innervation in the newborn dog. *Am. J. Physiol.* 243:H20–H26
28. Comline, R. S., Silver, M. 1966. The development of the adrenal medulla of the foetal and newborn calf. *J. Physiol.* 183:305–40
29. Su, C., Bevan, J. A., Assali, N. S., Brinkman, C. R. 1977. Development of neuroeffector mechanisms in the carotid artery of the fetal lamb. *Blood Vessels* 14:12–24
30. Falck, B., Hillarp, N.-A., Thieme, G., Torp, A. 1962. Fluorescence of catecholamines and related compounds condensed with formaldehyde. *J. Histochem. Cytochem.* 10:348–54
31. Cowen, T., Burnstock, G. 1980. Quantitative analysis of the density and pattern of adrenergic innervation of blood vessels. *Histochemistry* 66:19–34.
32. Su, C., Duckles, S. P., Florence, V. 1977. Uptake of ^{3}H-norepinephrine in rabbit mesenteric blood vessels. *Blood Vessels* 14:65–76
33. Hermann, W., Graefe, K.-H. 1977. Relationship between the uptake of ^{3}H metaraminol and the density of adrenergic innervation in isolated rat tissues. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 296:99–110
34. Duckles, S. P. 1980. Comparison of ^{3}H-norepinephrine and ^{3}H-metaraminol accumulation as indices of adrenergic nerve density in rabbit blood vessels. *Eur. J. Pharmacol.* 67:355–61
35. Cowen, T., Haven, A. J., Wen-Qin, C., Gallen, D. D., Franc, F., Burnstock, G. 1982. Development and aging of perivascular adrenergic nerves in the rabbit. A quantitative fluorescence histochemical study using image analysis. *J. Autonomic Nerv. Syst.* 5:317–36
36. Lundberg, J., Ljung, B., Stage, D., Dahlstrom, A. 1976. Postnatal ontogenic development of the adrenergic innervation pattern in the rat portal vein. *Cell Tissue Res.* 172:15–27
37. Todd, M. E. 1980. Development of adrenergic innervation in rat peripheral vessels: A fluorescence microscopic study. *J. Anatomy* 131:121–33
38. Gallen, D. D., Cowen, T., Griffith, S. G., Haven, A. J. Burnstock, G. 1982. Functional and non-functional nerve-smooth muscle transmission in the renal arteries of the newborn and adult rabbit and guinea pig. *Blood Vessels* 19:237–46
39. Dobrin, P. B. 1978. Mechanical properties of arteries. *Physiol. Rev.* 58:397–460
40. Berry, C. L., Looker, T., Germain, J. 1972. The growth and development of the rat aorta. I. Morphological aspects. *J. Anat.* 113:1–16
41. Looker, T., Berry, C. L. 1972. The growth and development of the rat aorta. II. Changes in nucleic acid and scleroprotein content. *J. Anat.* 113:17–34
42. Cox, R. H., Jones, A. W., Fischer, G. M. 1974. Carotid artery mechanics, connective tissue and electrolyte changes in puppies. *Am. J. Physiol.* 227:563–68
43. Cox, R. H., Jones, A. W., Swain, M. L. 1976. Mechanics and electrolyte composition of arterial smooth muscle in developing dogs. *Am. J. Physiol.* 231:77–83
44. Cox, R. H. 1981. Potassium efflux and chemical content of canine arteries during growth and development. *IRCS Med. Sci.* 9:820–21
45. Daniel, E. E., Paton, D. M., eds. 1975. *Methods in Pharmacology: Smooth Muscle, Vol. 3.* New York: Plenum. 731 pp.
46. Duckles, S. P., Silverman, R. W. 1980. Transmural nerve stimulation of blood vessels in vitro: A critical examination. *Blood Vessels* 17:53–57
47. Furchgott, R. F. 1972. The classification of adrenoceptors. In *Catecholamines,* ed H. Blaschko, E. Muscholl, pp. 283–335 New York: Springer-Verlag. 1054 pp.
48. Tallarida, R. J., Jacob, L. S. 1979. *The Dose-Response Relation in Pharmacology.* New York: Springer-Verlag 207 pp.
49. Williams, L. T., Lefkowitz, R. J. 1978. *Receptor Binding Studies in Adrenergic*

Pharmacology. New York: Raven. 151 pp.
50. Fleisch, J. H. 1980. Age-related changes in the sensitivity of blood vessels to drugs. *Pharmacol. Ther.* 8:477–87
51. Fleisch, J. H., Maling, H. M., Brodie, B. B. 1970. β-receptor activity in aorta. Variations with age and species. *Circ. Res.* 26:151–62
52. Fleisch, J. H., Hooker, C. S. 1976. The relationship between age and relaxation of vascular smooth muscle in the rabbit and rat. *Circ. Res.* 38:243–49
53. Herlihy, J. T., Yu, B. P. 1980. Dietary manipulation of age-related decline in vascular smooth muscle function. *Am. J. Physiol.* 238:H652–55
54. Cohen, M. L., Berkowitz, B. A. 1974. Age-related changes in vascular responsiveness to cyclic nucleotides and contractile agonists. *J. Pharmacol. Exp. Ther.* 191:147–55
55. Hayashi, S., Toda, N. 1978. Age-related changes in the response of rabbit isolated aortae to vasoactive agents. *Br. J. Pharmacol.* 64:229–37
56. Gray, S. D. 1977. Reactivity of neonatal canine aortic strips. *Biol. Neonate* 31:10–14
57. Wyse, D. G., Van Petten, G. R., Harris, W. H. 1977. Responses to electrical stimulation, noradrenaline, serotonin and vasopressin in the isolated ear artery of the developing lamb and ewe. *Can. J. Physiol. Pharmacol.* 55:1001–06
58. Ljung, B., Stage, D. 1975. Postnatal ontogenetic development of neurogenic and myogenic control in the rat portal vein. *Acta Physiol. Scand.* 94:112–27
59. Gray, S. D. 1976. Effect of angiotensin II on neonatal lamb carotid arteries. *Experientia* 32:350–51
60. Fehn, P. A., McCuskey, R. S. 1971. Response of the fetal mesenteric microvascular system to catecholamines. *Microvas. Res.* 3:104–09
61. Reddy, G. D., Gootman, N., Buckley, N. M., Gootman, P. M., Crane, L. 1974. Regional blood flow changes in neonatal pigs in response to hypercapnia, hemorrhage and sciatic nerve stimulation. *Biol. Neonate* 25:249–62
62. Buckley, N. M., Gootman, P. M., Gootman, N., Reddy, G. D., Weaver, L. C., Crane, L. A. 1976. Age-dependent cardiovascular effects of afferent stimulation in neonatal pigs. *Biol. Neonate* 30:268–79
63. Woods, J. R. Jr., Dandavino, A., Murayama, K., Brinkman, C. R. III, Assali, N. S. 1977. Autonomic control of cardiovascular functions during neonatal development and in adult sheep. *Circ. Res.* 40:401–07
64. Boatman, D. L., Shaffer, R. A., Dixon, R. L., Brody, M. J. 1965. Function of vascular smooth muscle and its sympathetic innervation in the newborn dog. *J. Clin. Invest.* 44:241–46
65. Gauthier, P., Nadeau, R. A., De Champlain, J. 1975. The development of sympathetic innervation and the functional state of the cardiovascular system in newborn dogs. *Can. J. Physiol. Pharmacol.* 53:763–76
66. Privitera, P. J., Loggie, J. M. H. Gaffney, T. E. 1969. A comparison of the cardiovascular effects of biogenic amines and their precursors in newborn and adult dogs. *J. Pharmacol. Exp. Ther.* 166: 293–98
67. Gootman, P. M., Gootman, N., Turlapaty, P. D. M. V., Yao, A. C., Buckley, B. J., Altura, B. M. 1981. Autonomic regulation of cardiovascular function in neonates. In *Development of the Autonomic Nervous System. Ciba Found. Symp. 83,* pp. 70–93. London:Pitman Medical
68. Fisher, D. J., Heymann, M. A., Rudolph, A. M. 1982. Regional myocardial blood flow and oxygen delivery in fetal, newborn and adult sheep. *Am. J. Physiol.* 243:H729–31
69. Buckley, N. M., Gootman, P. M., Yellin, E. J., Brazeau, P. 1979. Age-related cardiovascular effects of catecholamines in anesthetized piglets. *Circ. Res.* 45:282–92
70. Wilson, T. A., Kaiser, D. L., Wright, E. M., Peach, M. J., Carey, R. M. 1981. Ontogeny of blood pressure and the renin-angiotensin-aldosterone system. Sequential studies in the newborn lamb. *Circ. Res.* 49:416–23
71. Nelson, N. M. 1975. The onset of respiration. In *Neonatology: Pathophysiology and Management of the Newborn*, ed. G. Avery, pp. 76–97. Philadelphia: Lippincott. 1136 pp.
72. Rudolph, A. M. 1980. High pulmonary vascular resistance after birth. I. Pathophysiologic considerations and etiologic classification. *Clin. Pediatr.* 19:585–90
73. Leffler, C. S., Tyler, T. L., Cassin, S. 1978. Effect of indomethacin on pulmonary vascular response to ventilation of fetal goats. *Am. J. Physiol.* 234:346–51
74. Tooley, W. J., Phibbs, R. H. 1975. Delivery room management of the newborn. See Ref. 71, pp. 111–26
75. Ahlquist, R. P., Huggins, R. A., Woodbury, R. A. 1947. The pharmacology of

benzyl-imidazoline (Priscol). *J. Pharmacol. Exp. Ther.* 89:271–88
76. Goetzman, B. W., Milstein, J. M. 1979. Pulmonary vasodilator action of tolazoline. *Pediat. Res.* 13:942–44
77. Tucker, A., Brown, D. T., Greenlees, K. H. 1982. Pulmonary and systemic vascular actions of tolazoline in anesthetized dogs. *Ped. Pharmacol.* 2:231–43
78. Volpe, J. J. 1979. Intracranial hemorrhage in the newborn: current understanding and dilemmas. *Neurology* 29:632–35
79. Lou, H. C., Skov, H., Pedersen, H. 1979. Low cerebral blood flow: A risk factor in the neonate. *J. Pediatr.* 95:606–09
80. Lou, H. C., Lassen, N. A., Tweed, W. A., Johnson, G., Jones, M., Palahniuk, R. J. 1979. Pressure passive cerebral blood flow and breakdown of the blood-brain barrier in experimental fetal asphyxia. *Acta Paediatr. Scand.* 68:57–63
81. Wigglesworth, J. S., Pape, K. E. 1978. An integrated model for haemorrhagic and ischaemic lesions in the newborn brain. *Early Hum. Dev.* 212:179–99
82. Papile, L., Burstein, J., Burstein, R., Koffler, H. 1978. Incidence and evolution of subependymal and intraventricular hemorrhage. *J. Pediatr.* 92:529–34
83. Goddard, J., Lewis, R. M., Alcala, H., Zeller, R. S. 1980. Intraventricular hemorrhage: An animal model. *Biol. Neonate* 37:39–52
84. Lou, H. C., Lassen, N. A., Friis-Hansen, B. 1970. Impaired autoregulation of cerebral blood flow in the distressed newborn infant. *J. Pediatr.* 94:118–21
85. Volpe, J. J. 1979. Cerebral blood flow in the newborn infant: Relation to hypoxic-ischemic brain injury and periventricular hemorrhage. *J. Pediatr.* 94:170–73
86. Lou, H. C., Lassen, N. A., Friis-Hensen, B. 1979. Is arterial hypertension crucial for the development of cerebral hemorrhage in premature infants? *Lancet* 1:1215–17
87. Touloukian, R. J. 1980. Etiologic role of the circulation. In *Neonatal Necrotizing Enterocolitis*, ed. E. G. Brown, A. Y. Sweet, pp. 41–56. New York: Grune & Stratton. 203 pp.
88. Brown, E. G., Sweet, A. Y. 1982. Neonatal necrotizing enterocolitis. *Pediatr. Clin. North Am.* 29:1149–70
89. Ryder, R. W., Shelton, J. D., Gwinan, M. E. 1980. Necrotizing enterocolitis: A prospective multicenter investigation. *Am. J. Epidemiol.* 112:113–23
90. James, L. S. 1959. Biochemical aspects of asphyxia at birth. In *Adaptation to Extrauterine Life: Report of the 31st Ross Conference on Pediatric Research*, ed. T. K. Oliver, Jr. pp. 66–71. Columbus: Ross Lab. 94 pp.
91. Scholander, P. E. 1963. The master switch of life. *Sci. Am.* 209:92–105