Sympathetic Innervation of the Developing Rabbit Heart
BIOCHEMICAL AND HISTOCHEMICAL COMPARISONS OF FETAL, NEONATAL, AND ADULT MYOCARDIUM
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ABSTRACT
The sympathetic innervation of the rabbit heart, as a function of age, was studied by measuring the cardiac concentration of catecholamines and observing the anatomic distribution of sympathetic nerves by the monamine fluorescence technique. The cardiac concentration of norepinephrine in late gestation was quite low; the levels rose progressively after birth to reach adult levels by about three weeks of age. Similar small amounts of epinephrine were found in the hearts at all ages. Substantially less change in adrenal catecholamines accompanied advancing age. At all ages a close correlation was noted between the norepinephrine levels and the histochemical demonstration of sympathetic innervation. Intensely fluorescent, terminal varicosities were observed within large preterminal nerve trunks only in the youngest animals, suggesting that the sympathetic nerves move into, rather than form within, the heart. Chromaffin cells were observed in the hearts at all ages.

ADDITIONAL KEY WORDS
norepinephrine epinephrine adrenal chromaffin cells myocardial catecholamines

Although the adrenergic nervous system plays an important role in the control of cardiac contractility in the mature mammal, its significance in the perinatal period is not clear. Physiologic and pharmacologic studies undertaken to assess the maturation of the autonomic control of the circulation have been largely concerned with the ability of young animals to respond to various physiologic stimuli, such as hypoxemia and carotid sinus hypotension, or to the injection of catecholamines (1). A number of observations in rabbits of varying ages suggest that the circulatory control of the newborn is under no (2), a lesser (3), or a comparable (1) degree of neural control, as compared to the adult. The development of the separate factors constituting an integrated circulatory response—the afferent, central, and efferent components of a vascular reflex, the responsiveness of the peripheral vasculature, and the direct inotropic and chronotropic effects on the myocardium—have not yet been analyzed quantitatively.

The objective of the present investigation was to define more clearly the development of sympathetic innervation of the rabbit heart. The cardiac concentration of norepinephrine in fetal, neonatal, and adult animals was employed as an index of the maturity of sympathetic innervation because the heart's stores of norepinephrine are localized almost exclusively in intracellular storage sites within the terminations of the sympathetic nerves (4). In addition, the monamine fluorescence technique of Falck and Owman (5) was em-
Catecholamine fluorescent nerve fibers in the adult rabbit atrium (A) and ventricle (B) and in the two-week-old rabbit atrium (C) and ventricle (D). Note innervation to coronary artery (arrow) (×170). The fluorescence is equally intense at both ages while the density of adrenergic nerve fibers appears somewhat greater in the adult.
Sparse, small nerve terminals and several large preterminal nerve trunks (arrows) in the two-day-old rabbit ventricle (A). Few small fluorescent fibers and preterminal nerve trunks (arrows) in the atrium (B) and ventricle (C) of a 29-day-old rabbit fetus (X170).

In the adult, the heart is innervated by a dense network of intensely green-fluorescent varicose nerve fibers (Fig. 2, A and B). These beaded, terminal ramifications of sympathetic nerves take the form of a typical autonomic ground plexus. At ages 3 to 5 weeks, the hearts contained essentially the same density of nerve fibers as that seen in the adult rabbits. At the age of 2 weeks, however, the
Atrium of a 29-day-old rabbit fetus. Very large nerve trunk containing abundant green fluorescent varicosities (×420).

Clusters of chromaffin cells adjacent to the aorta (A) (×420) and in the atrium (B) (×650) of a 4-day-old rabbit.
myocardium is only moderately innervated by catecholamine-containing neurons (Fig. 2, C and D), and at 2 days of age only a sparse distribution of fluorescent nerve fibers was observed (Fig. 3, A). Innervation was least dense in the fetal hearts (Fig. 3, B and C). The hearts of all of the animals up to 2 weeks of age contained large, intensely fluorescent, preterminal nerve trunks in the epicardium, in the tunica adventitia surrounding coronary arteries, and coursing in the connective tissue between cardiac muscle bundles (Figs. 3 and 4). In these younger animals intense fluorescence resided only in terminal varicosities within the large nerve trunks. In contrast, the nerve trunks in the older rabbits did not contain terminal varicosities and fluoresced only weakly.

In addition to the catecholamine-containing nerve processes and terminals, the rabbit atria at all ages contained intensely yellow-green fluorescent chromaffin cells (Fig. 5). These cells were most numerous in the connective tissue between the aorta and pulmonary artery at the level of the aortic valves, or in close proximity to nonfluorescent parasympathetic ganglion cells. The chromaffin cells were usually arranged in clusters and appeared to be interconnected.

Discussion

The heart of the adult mammal is richly supplied with sympathetic nerves, and the release of norepinephrine from the endings of these nerves provides one of the fundamental mechanisms for the modulation of cardiac contractility (9). Although there is evidence to suggest that regulation of the heart rate and arterial blood pressure of the newborn rabbit is under some autonomic control (10, 11), the extent to which sympathetic nerves contribute to the contractile state of the heart at this stage of development has not been previously defined. The results of the present investigation demonstrate a marked paucity of sympathetic nerves and low norepinephrine stores in the fetal and newborn rabbit heart as compared to the adult. Recently, Glowinski et al. (12) studied the uptake and retention of labeled norepinephrine in the developing rat and provided evidence suggesting that either the extent of myocardial sympathetic innervation had not reached adult levels in the newborn or the sympathetic nerves in the newborn are unable to take up and bind norepinephrine. The latter explanation appears unlikely in view of the present findings. The histological demonstration of the ingrowth of large preterminal nerve trunks enclosing intensely fluorescent, catecholamine-containing terminal varicosities suggests that the latter move into the heart during early development and branch out to form the autonomic ground plexus. Moreover, it appears that a significant proportion of the norepinephrine measured in the fetal and newborn heart resides in the preterminal nerve trunks and therefore may not be in close anatomic proximity to the adrenergic receptor of the myocardial cell.

Conflicting statements exist concerning the influence of innervation, per se, on the responsiveness of the developing heart to norepinephrine. Several workers have reported that the establishment of innervation endows the embryonic heart with a markedly increased sensitivity to the adrenergic neurotransmitter (13), although others have not confirmed these observations (14). Similarly, until recently it had not been determined in the mammal whether myocardial receptor sites for the adrenergic neurotransmitter were fully functional before or after the establishment of an extrinsic nerve supply. Recent studies from this laboratory on isolated fetal myocardium from lambs demonstrated that the functional maturity of the heart's beta-receptors precedes the complete development of an extrinsic sympathetic nerve supply (unpublished observations).

At a comparable stage of development the adrenal glands, unlike the heart, contain abundant catecholamine stores. It has been shown that the ordinary activity of the adrenergic nervous system may have minimal effects on the normal heart and that the intrinsic contractile state of the myocardium may not be influenced by alterations in en-
SYMPATHETIC INNERVATION OF RABBIT HEARTS

The force of contraction of the heart may be stimulated profoundly by an increase in the number of impulses traversing the sympathetic nerves whenever an imbalance exists between the cardiac output and the perfusion requirements of the peripheral tissues (9). When the latter occurs in the perinatal period, the interaction between a supersensitive myocardium and the adrenal release of catecholamines may play a more critical, compensatory, role in maintaining ventricular contractility than in the adult. In adult animals it is clear that only a small fraction of the normal cardiac store of norepinephrine is necessary to elicit a functional response to tyramine (18) or adrenergic nerve stimulation (17) although the magnitude of the response may be reduced (18).

Catecholamine-containing cells that resemble the chromaffin cells of the adrenal medulla have been identified previously within the hearts of some mammalian species (19). The present investigation demonstrates their presence in rabbit myocardium and even in the fetus. Unlike the extra-adrenal chromaffin tissue of the preaortal paraganglia (organs of Zuckerkandl), which contains norepinephrine exclusively and undergoes postnatal involution (20), chromaffin cells were observed in the rabbit hearts at all of the ages studied. It has been suggested that hypoxia and hypocapnia may directly stimulate the release of catecholamines from the preaortal paraganglia during the perinatal period (20). Recently, it has been suggested that chromaffin cells may serve to modulate cholinergic transmission through the cardiac ganglia (19). It is apparent, however, that the precise function of myocardial extra-adrenal chromaffin tissue awaits more detailed investigation.

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