Distribution of Norepinephrine in the Failing Bovine Heart
CORRELATION OF CHEMICAL ANALYSIS AND FLUORESCENCE MICROSCOPY

By John H. K. Vogel, M.D., David Jacobowitz, Ph.D., and Charles A. Chidsey, M.D.

ABSTRACT

Studies were carried out during experimental heart failure induced in calves to compare the depletion of cardiac norepinephrine in the failing heart to the histochemical appearance of the adrenergic innervation in this tissue. A correlation has been established between the reduction of norepinephrine concentration and the changes in the distribution of the adrenergic neurotransmitter in the tissues. Absence of fluorescence in terminal varicose fibers in close association with cardiac muscle cells was characteristic of the failing heart. Little or no change occurred in preterminal fibers or in terminal fibers in connective tissue septa or around blood vessels. In two steers, recovery from heart failure was shown to be associated with a virtual restoration of the norepinephrine concentration and of the histochemical appearance of the adrenergic nerve distribution in 28 days or less. These findings suggest that a reversible abnormality, possibly of neurotransmitter storage or synthesis, or both, is induced in the terminal portion of the cardiac adrenergic innervation as the result of the development of the failure state.

ADDITIONAL KEY WORDS chromaffin cells mast cells adrenergic nerves fluorescent histochemistry myocardial innervation calves

A marked reduction in norepinephrine concentration has been observed in the failing heart of both man (1) and experimental animals (2). Previous biochemical and pharmacological studies have suggested that this reduction was associated with a significant functional impairment of the cardiac sympathetic nerves, involving both storage (3) and synthesis (4) of the neural transmitter substance. The importance of this biochemical abnormality has been demonstrated by the observed depression of the response to stimulation of the postganglionic nerves supplying the failing heart (5). However, in spite of these studies demonstrating the functional significance of this abnormality, the mechanisms which may be responsible for its development remain largely unexplained.

The present study was undertaken to define the microanatomical distribution of the adrenergic nerves in control, hypertrophied, and failing hearts and to relate these observations with chemical measurements of the total adrenergic neurotransmitter contained within the myocardial tissue. In addition, studies were carried out in hearts following recovery from the failure state. For this purpose a bovine experimental heart failure preparation was used (6). In this study a correlation had been observed between the depletion of total...
tissue norepinephrine determined chemically and the reduction of catecholamine within adrenergic nerves in the failing heart. Furthermore, the microanatomical abnormality of the cardiac adrenergic nerves appeared to be oriented principally around terminal nerve endings, suggesting a localized dysfunction of the nerves in their terminal varicose portions. When animals were studied after recovery from heart failure, both the microanatomical and the chemical abnormalities had returned virtually to the control condition.

Methods

Right pulmonary artery ligation was performed within 48 hours after birth in 17 calves which were born at 5,280 ft. This operation resulted in progressive pulmonary hypertension with right ventricular hypertrophy and subsequent development of heart failure (6). The pulmonary artery ligations were performed through a right thoracotomy at the level of the fourth intercostal space using halothane\(^1\) and nitrous oxide anesthesia. The branches of the right pulmonary artery to the upper, middle, and lower lobes were ligated individually, care being taken to avoid any manipulation of the great vessels or the pericardium. The animals recovered from the operative procedure, and at a later time hemodynamic studies were carried out, both before and after the development of heart failure. Cardiac catheterization was performed via an external jugular vein with the animals standing awake and unsedated (6). Ten animals were studied prior to failure; four at 3 days, three at 10 days, and three at 28 days. Three calves were studied within 1 week after the onset of failure (early heart failure); four calves were studied after having been in failure for more than 1 week (late heart failure). Heart failure was considered to be present when persistent distention of the external jugular veins was observed. This initial manifestation of failure was followed by progressive fluid accumulation in the animals leading to obvious ascites and distention of the brisket.

We have previously shown that left pulmonary artery ligation in calves at sea level does not result in progressive pulmonary hypertension (6). However, when such animals are brought to Denver (5,280 ft) progressive pulmonary hypertension and heart failure will occur in these animals. This hemodynamic sequence can be reversed by returning the hypertensive animal with right heart failure to sea level. Using this preparation we were able to obtain observations in 36-month-old steers both in severe heart failure and following recovery from late heart failure effected by returning the animals with heart failure to sea level. Four steers that had failed to develop pulmonary hypertension following left pulmonary artery ligation at sea level were transported to Denver where they developed progressive pulmonary hypertension and congestive heart failure. Two of these were killed after they had demonstrated evidence of failure for 1 month. Two others, which had also been in failure for 1 month, were returned to sea level for 10 and 28 days, respectively, and then killed. Two unoperated animals of a comparable age were killed as controls.

Following the hemodynamic measurements, the animals were killed and the hearts removed for chemical and histochemical determinations. The hearts were placed on crushed ice for sectioning. Tissues for norepinephrine determination were frozen on dry ice and later assayed by a fluorometric method (7, 8). Tissues for fluorescence microscopy were rapidly removed from each cardiac chamber and immediately frozen in isopentane cooled by liquid nitrogen. Subsequently, these specimens were freeze-dried at —30°C, treated with stock paraformaldehyde vapor at 80°C for 1 hour, embedded in paraffin under vacuum, and sectioned at 14 µm for fluorescence microscopy according to the method of Falck and Hillarp (9-11). The specificity of this method has been demonstrated for norepinephrine, epinephrine, and dopamine, which give a green fluorescence, as distinct from 5-hydroxytryptamine, which gives a yellow fluorescence (10). Although quantification is not possible with this technique, one can roughly estimate whether an experimental procedure has caused an increase, decrease, or no change in neuronal content of monoamines. For purposes of quantification, the standard chemical assays were used. After removal of the myocardial specimens, the hearts were stripped of atria, fat, great vessels, and valve tissue, and the individual weights of the free wall of the right ventricle and left ventricle with septum were determined.

Results

CHEMICAL STUDIES OF NOREPINEPHRINE

Normal Hearts.—The concentrations of norepinephrine in the heart were similar in the control animals at ages varying from birth to 18 months. Little change was found in the norepinephrine concentration in these tissues between birth and 18 months, but the nor-
NOREPINEPHRINE IN THE FAILING HEART

TABLE 1

Normal Bovine Cardiac Norepinephrine (μg/g)

<table>
<thead>
<tr>
<th>Age</th>
<th>Right atrium</th>
<th>Right ventricle</th>
<th>Left atrium</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 day</td>
<td>1.36</td>
<td>1.47</td>
<td>1.99</td>
<td>1.48</td>
</tr>
<tr>
<td></td>
<td>0.79</td>
<td>0.94</td>
<td>0.76</td>
<td>1.00</td>
</tr>
<tr>
<td>2 weeks</td>
<td>1.81</td>
<td>1.80</td>
<td>1.21</td>
<td>0.88</td>
</tr>
<tr>
<td></td>
<td>2.36</td>
<td>2.08</td>
<td>2.90</td>
<td>1.19</td>
</tr>
<tr>
<td>4 weeks</td>
<td>1.12</td>
<td>1.25</td>
<td>1.08</td>
<td>0.97</td>
</tr>
<tr>
<td></td>
<td>1.43</td>
<td>1.76</td>
<td>1.31</td>
<td>1.28</td>
</tr>
<tr>
<td>8 weeks</td>
<td>1.30</td>
<td>1.12</td>
<td>1.50</td>
<td>0.94</td>
</tr>
<tr>
<td></td>
<td>1.59</td>
<td>1.38</td>
<td>1.71</td>
<td>1.29</td>
</tr>
<tr>
<td></td>
<td>1.44</td>
<td>1.69</td>
<td>1.63</td>
<td>1.44</td>
</tr>
<tr>
<td>MEAN*</td>
<td>1.47</td>
<td>1.50</td>
<td>1.57</td>
<td>1.16</td>
</tr>
<tr>
<td>18 months</td>
<td>1.13</td>
<td>1.46</td>
<td>1.26</td>
<td>1.11</td>
</tr>
<tr>
<td></td>
<td>1.76</td>
<td>0.87</td>
<td>1.44</td>
<td>0.64</td>
</tr>
<tr>
<td>MEAN</td>
<td>1.44</td>
<td>1.16</td>
<td>1.35</td>
<td>0.88</td>
</tr>
<tr>
<td>TOTAL MEAN</td>
<td>1.46</td>
<td>1.44</td>
<td>1.53</td>
<td>1.11</td>
</tr>
<tr>
<td>SE</td>
<td>0.13</td>
<td>0.11</td>
<td>0.17</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Mean of all measurements up to and including those at 8 weeks.

Noradrenaline concentration was lower in the ventricles of the 36-month-old animals (Tables 1 and 2). Except in the animals killed immediately after birth, right ventricular norepinephrine tended to be higher than the values determined in the left ventricle.

Hypertrophy.—Progressive hypertrophy of the right ventricle was observed in those animals in which right ventricular hypertension had been induced, both before and after the development of failure. The right ventricular portion of the total ventricular weight $RV/TV \times 100$) was increased in the animals before the onset of failure from a control value of $33 \pm 0.7\%$ (average right ventricular pressure 48/5 mm Hg) to $38 \pm 2.3\%$ at 10 days (right ventricular pressure 90/7 mm Hg), and $42 \pm 2.3\%$ at 28 days (right ventricular pressure 133/12 mm Hg). Three days following surgery it was $32 \pm 1.2\%$ (right ventricular pressure 78/8 mm Hg), which was not significantly different from the controls. There was only a minimal alteration in cardiac norepinephrine concentration in these hypertrophied nonfailing hearts (Fig. 1). The right ventricular norepinephrine concentration was slightly reduced at 10 days, averaging 0.75 μg/g. However, by 28 days this value had increased to nearly normal levels, averaging 1.16 μg/g.

Heart Failure.—In the animals with failure, both early and late, a persistent increase in the right ventricular percentage of total ventricular weight was noted, averaging $48 \pm 2.7\%$ in early failure (right ventricular pressure 94/22 mm Hg) and $52 \pm 3\%$ in late failure (right ventricular pressure 104/30 mm Hg). In both stages of failure, cardiac norepinephrine concentration was decreased (Fig. 1). There was an obviously greater decrease in the right ventricular and right atrial values than in those of the left atrium and ventricle. A more striking depletion of norepinephrine was observed in the late stage of failure in the right ventricle; mean values were 0.11 μg/g in late failure compared to 0.38 μg/g in early failure. Similar differences between these two stages of failure were observed in the other cardiac chambers.

Recovery from Failure.—The norepinephrine concentrations in the hearts of two of the 36-month-old animals, killed after demonstrating evidence of failure for 1 month, were greatly reduced (Table 2) compared to the values observed in the two control animals.

TABLE 2

Cardiac Norepinephrine (μg/g) in Thirty-Six-Month-Old Steers

<table>
<thead>
<tr>
<th></th>
<th>Right atrium</th>
<th>Right ventricle</th>
<th>Left atrium</th>
<th>Left ventricle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control Steers</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>1.26</td>
<td>0.58</td>
<td>1.48</td>
<td>0.58</td>
</tr>
<tr>
<td>No. 2</td>
<td>1.09</td>
<td>0.60</td>
<td>1.19</td>
<td>0.86</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>1.18</td>
<td>0.59</td>
<td>1.34</td>
<td>0.72</td>
</tr>
<tr>
<td><strong>Steeers in Failure</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. 1</td>
<td>0.32</td>
<td>0.16</td>
<td></td>
<td>0.40</td>
</tr>
<tr>
<td>No. 2</td>
<td>0.34</td>
<td>0.12</td>
<td>0.78</td>
<td>0.29</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>0.33</td>
<td>0.14</td>
<td>0.78</td>
<td>0.34</td>
</tr>
<tr>
<td><strong>Steeers after Recovery</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10 days</td>
<td>0.43</td>
<td>0.33</td>
<td>1.23</td>
<td>0.65</td>
</tr>
<tr>
<td>28 days</td>
<td>1.41</td>
<td>0.52</td>
<td>1.41</td>
<td>0.44</td>
</tr>
<tr>
<td><strong>MEAN</strong></td>
<td>0.92</td>
<td>0.42</td>
<td>1.32</td>
<td>0.54</td>
</tr>
</tbody>
</table>

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Mean values and SEM (indicated by bar) for cardiac norepinephrine concentrations (ordinate) in control calves (C); at 3, 10, and 28 days after right pulmonary artery ligation; and in early (E) and late (L) congestive heart failure (CHF). RA = right atrium, LA = left atrium, RV = right ventricle, and LV = left ventricle.

recovery from heart failure had been effected by return to sea level for 10 and 28 days, respectively, the cardiac norepinephrine was clearly elevated above the levels observed in failure, but was not quite as high as the levels observed in the hearts of the two control 36-month-old steers (Table 2). The steer that took 28 days to recover was essentially normal.

HISTOCHEMICAL OBSERVATIONS
Normal and Hypertrophied Calf Hearts

The histochemical appearance of the adrenergic innervation was essentially the same in both normal and hypertrophied calf hearts. All catecholamine-containing fibers had a green fluorescence specifically characteristic of norepinephrine and epinephrine.

Atria.—Both atria contain a large number of varicose nerve fibers with an intense fluorescence (Fig. 2). Fine varicose nerve terminals were seen in intimate contact with cardiac muscle fibers. In addition, larger pre-terminal nerve trunks containing varicose fibers were present in connective tissue regions within the heart and in close proximity to blood vessels (Fig. 3, A). The smooth muscle of the coronary arteries was surrounded by a dense network of adrenergic nerve terminals (Fig. 2, B). There was no apparent difference between the nerves to the right and left atria. However, the A-V nodal region within the interatrial septum contained a dense network of catecholamine fluorescent nerves (Fig. 2, C). In addition, many clusters of intense green-yellow fluorescent cells were observed in the interatrial septum close to nonfluorescent (cholinergic) ganglion cells. These fluorescent cells strongly resemble the chromaffin cells of the adrenal medulla in size, shape, color, and intensity of fluorescence. In the atria, they are primarily in the interatrial septum closely associated with ganglion cells and blood vessels. They are usually found in clusters of approximately 5 to 15 cells.

Ventricles.—The ventricles contain a large number of fluorescent nerve fibers similar in density and distribution to those described above for the atria (Figs. 2, D and 3, A). In both ventricles the bundle branches of the ventricular conducting system can be readily
FIGURE 2
A: Left atrium, normal calf. Adrenergic varicose nerve fibers are observed coursing over the cardiac muscle cells. Smooth autofluorescent elastic fibers are also observed ($\times 170$). B: Coronary artery, normal calf left atrium. Adrenergic nerve fibers following the course of a branching coronary artery. Note dimly fluorescent preterminal nerve trunk (arrow) ($\times 170$). C: Calf A-V node. Dense innervation with adrenergic nerves ($\times 170$). D: Right ventricle, normal calf. Adrenergic fibers are similar in distribution as in the atria ($\times 170$).

identified using the fluorescence microscope. This tissue is essentially devoid of the diffuse faint-green autofluorescence observed in the cardiac muscle. However, autofluorescent
A: Large preterminal nerve trunk in right ventricle. Note varicose fibers within the trunk (×170). B: A bundle branch of the atrioventricular conducting system (AVC) of the calf. Note adrenergic fibers coursing within the surrounding connective tissue sheath. Note also fluorescent mast cells (arrow) (×170). C: Nonfluorescent cholinergic ganglion cells (G) in the epicardium of the left ventricle of the calf. Fluorescent terminal varicosities (white arrows) appear to make contact with the ganglion cells. A terminal nerve fiber (black arrow) is seen emanating from a nerve trunk below (×260). D: Green-yellow fluorescent chromaffin cells in the epicardial region of the left ventricle of a calf in heart failure. These cells were close to a ganglion (×660).
Calf, early heart failure (×170). A: Left atrium. Similar to normal atrium. Moderate number of nerve terminals compared to left atrium. Normal appearing preterminal nerve trunks (PT) in connective tissue (CT) region. Note fluorescent mast cells (arrows). C: Left ventricle. Similar to normal ventricle. D: Right ventricle. Markedly reduced content of nerve terminals with diminished intensity of fluorescence. Preterminal nerves are present as in normal ventricle.

orange granules are present in some of the cells of this tissue. The identity of these granules is unknown. The conduction bundles are surrounded by green autofluorescent con-
FIGURE 5

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FIGURE 6

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nective tissue containing elastic fibers. Frequently, adrenergic fibers can be seen coursing within the surrounding connective tissue sheath, but no nerve fibers ramify or terminate upon the individual cells of the bundle branches of the conducting system (Fig. 3,
B). In one calf, nonfluorescent ganglion cells were observed in the epicardial connective tissue of the left ventricle (Fig. 3, C). Several of the cells appeared to be innervated by adrenergic nerve terminals. In another calf (with heart failure, described below) nonfluorescent ganglion cells were found in the epicardial region of the left ventricle. In close proximity to these was a large cluster of green-yellow fluorescent chromaffin cells (Fig. 3, D). In addition, a large, smooth, green fluorescent preterminal nerve trunk was observed in the confines of the ganglion.

In both atria and ventricles an abundant number of intense green fluorescent mast cells were present between the muscle fascicles within the connective tissue (Figs. 3, B; 4, B; 5, B and C; and 6, B). Green fluorescent distinct granules were usually observed surrounding a nonfluorescent nucleus.

Heart Failure

Early Failure.—In the left atrium the number of fluorescent nerve fibers and the intensity of their fluorescence were essentially similar to those of the normal calf atrium (Fig. 4, A). The right atrium contained only a moderate number of fluorescent nerve terminals, i.e., much fewer than the left atrium (Fig. 4, B). The intensity of fluorescence was reduced in many of the fibers. However, the large preterminal nerve trunks in the connective tissue regions appeared to be normal in number and intensity of fluorescence. In the left ventricle the content of fluorescent nerves and intensity of their fluorescence appeared to be normal (Fig. 4, C). The right ventricle contained a markedly reduced content of fluorescent terminals whose intensity of fluorescence was diminished (Fig. 4, D). Here too, preterminal nerve trunks present in the intermuscular connective tissue appeared normal. Green fluorescent mast cells were present in all the chambers of the heart. No unusual changes in the catecholamine content of these cells were noted.

Late Failure.—A moderate-to-marked reduction of the fluorescent terminals and the intensity of their fluorescence was seen in the left atrium (Fig. 5, A). In all specimens, those nerves contained within the connective tissue septa between the muscle bundles were usually much more intensely fluorescent than those nerve terminals within close proximity to the muscle cells. Large intense fluorescent preterminal nerve trunks were present primarily in connective tissue regions. Nerve fibers of normal intensity of fluorescence were observed surrounding the coronary arteries between the tunica adventitia and tunica media. In the right atrium the number of fluorescent fibers and the intensity of their fluorescence was very low (Fig. 5, B). Some large preterminal nerve trunks containing varicose fibers were observed in connective tissue regions and in close proximity to blood vessels. In the left ventricle a marked reduction in the number of fluorescent nerve fibers and the intensity of their fluorescence was observed (Fig. 5, C). Some nerves with normal intensity of fluorescence were present primarily in the connective tissue spaces. Blood vessels were generally innervated with nerve fibers having normal fluorescence. Large, smooth, fluorescent preterminal nerve trunks were present in connective tissue regions and adjacent to blood vessels. The A-V conducting bundle branches showed no unusual histological changes. The orange fluorescent granules were still present and unaltered in content or intensity of fluorescence. Terminal varicose fibers were present in the connective tissue surrounding the bundle branches. In the right ventricle the number of fluorescent nerve fibers and the intensity of their fluorescence was markedly reduced (Fig. 5, D). However, the blood vessels contained many intensely fluorescent fibers surrounding the tunica media. Large, smooth, preterminal nerve trunks were present primarily in the connective tissue septa and these demonstrated fluorescence of moderate intensity.

Studies Carried Out After Recovery from Heart Failure in Steers

The histochemical picture of the myocardium of control steers was similar to that observed in control calves except that there were fewer fluorescent nerve fibers in the ven-
tricles. In addition, there were fewer fluorescent preterminal nerve trunks.

The adrenergic nerves in the heart of the steer studied 10 days after beginning recovery from failure demonstrated a substantial increase in both fluorescence intensity and number of fluorescent terminal varicosities in the right atrium and ventricle, whereas the left atrium and ventricle appeared essentially normal. In the steer studied 28 days after beginning recovery from failure, the adrenergic nerves in all four chambers appeared essentially normal (Fig. 6).

**Discussion**

In these studies a comparison has been made between alterations in myocardial norepinephrine concentration determined chemically and changes in the fluorescence of adrenergic nerves during the development of heart failure in calves with experimentally induced pulmonary hypertension. Our findings have demonstrated a correlation between the depletion of myocardial norepinephrine and changes in catecholamine fluorescence (9-11). Little or no chemical or histochemical change was seen in the hypertrophied heart prior to the onset of hemodynamic evidence of failure. These findings are in contrast to observations of a striking depletion of cardiac norepinephrine in the nonfailing hypertrophied ventricle reported by Spann et al. (12). However, in their studies, marked right ventricular hypertension was produced acutely by surgical constriction of the main pulmonary artery, and it is entirely possible that this procedure may have led to ischemic injury of the right ventricular myocardium (13) and of the sympathetic innervation of the heart. With the onset of failure, we observed both a depletion of chemically determined norepinephrine and a marked reduction in the number of fluorescent terminal adrenergic nerves in close proximity to the cardiac muscle cells with an absence of the fine varicosities characteristic of the adrenergic ground plexus. However, these varicose terminal nerve fibers were unchanged in intermuscular septal areas and also in areas surrounding blood vessels. In early failure the changes in both norepinephrine concentration and adrenergic nerve distribution were less striking than in the later stages of failure, suggesting that failure of cardiac performance precedes, and is not the result of, norepinephrine depletion in the heart. In early failure fluorescent terminal fibers were decreased in some muscle bundles, whereas in late failure there was a greater reduction in all muscle bundles. The absence of fluorescent neurons does not necessarily indicate an anatomic loss of neural element, since it can also be explained by metabolic dysfunction of the neuron, with subsequent diminution of stored norepinephrine within the adrenergic terminal.

Mast cells with an intense green fluorescence were observed in all chambers of these hearts. There was no obvious alteration in the intensity of fluorescence or number of these cells in any stage of heart failure examined in this study. Previous investigations of mast cells in ruminant tissue have demonstrated that the distribution of these cells corresponds with the occurrence of dopamine (14). The functional role of these mast cells in the heart is unclear. It is recognized that dopamine is an effective beta-receptor agonist with inotropic properties (15). The absence of notable structural abnormality of these cells suggests that the mechanisms responsible for the loss of fluorescence in the neuronal tissue do not similarly affect these dopamine containing cells.

The calf and steer are one of the few species that has parasympathetic ganglion cells in both the ventricles and atria. Both adrenergic terminal varicosities and clusters of chromaffin cells were present in close proximity to ganglion cells. This is consistent with observations in several other species (16). The release of norepinephrine from these endings and chromaffin cells could result in a depression of ganglionic transmission and therefore serve as a possible modulatory influence on cholinergic transmission through the ganglion. It has been well documented that catecholamines are capable of depress-
It is of interest that although nodal tissue has a dense adrenergic innervation, the bundle branches of the atrioventricular conducting system were essentially devoid of sympathetic nerves. However, a considerable number of nerves are present within the connective tissue immediately surrounding the bundle branches within the ventricles. This arrangement is reminiscent of sympathetic nerves within the tunica adventitia of arteries in close apposition to the muscle (tunica media) and suggests a possible influence of adrenergic nerves on the atrioventricular conducting system. No apparent change in the catecholamine content of this adrenergic innervation was observed in the failing heart. It was somewhat unexpected from previously reported observations (20) that the cardiac adrenergic innervation was essentially completely established in our newborn calves, showing no further development in the early period of body growth.

The changes in the adrenergic innervation of the failing heart, which were noted predominantly in the terminal varicose fibers in close approximation to muscle bundles, may indicate that these fibers were influenced in some manner by an alteration taking place at the region of close association between the nerve terminal and the cardiac muscle cell. This histochemical picture is similar to that observed in iris tissue removed from animals pretreated with reserpine in which the neurotransmitter store was partially repleted by administration of norepinephrine. In these tissues there was a marked reduction in fluorescence of terminal varicosities, with lesser involvement of the preterminal nerves (21). The alteration in these reserpinized tissues was presumed to be due to interference with an ATP-Mg$^{2+}$-dependent mechanism for the binding of amines. However, there is some question whether a deficit in myocardial ATP is present in the failing heart. Thus, surgical specimens obtained from failing human hearts reveal no change in ATP or creatine phosphate concentrations and mitochondria prepared from failing hu-

man hearts have normal oxidative phosphorylation (22). Although a minor reduction in creatine phosphate has been observed in experimental heart failure, other variables relating to energy production are normal (23). However, in spite of the lack of evidence suggesting a defect in energy production in the tissue as a whole, localized areas of hypoxia may occur in the myocardium as a result of hypertrophy and dilation of the failing ventricle (24). Metabolic derangements associated with such hypoxic areas may lead to a substantial regional alteration of neural function. One possible metabolic derangement might involve the production of an abnormal intermediate in the biosynthesis of norepinephrine. Thus, 6-hydroxydopamine has been shown both to deplete norepinephrine from adrenergic nerves and actually to alter neural structure (25).

In these studies we have demonstrated not only that a marked alteration in the adrenergic innervation occurs in the failing myocardium, but also that with recovery from heart failure reconstitution occurs. Substantial reconstitution of the normal pattern of adrenergic nerves occurred as early as 10 days after returning the animals to an environment where cardiac compensation was again possible. We cannot determine from these observations whether the process of recovery involved a restoration of anatomically absent nerve terminations in the substance of the muscle bundles or a recovery of intraneuronal function necessary for the storage and synthesis of a normal complement of neurotransmitter substance in the terminations. It is traditionally accepted that nerve regrowth occurs at the rate of approximately 1 mm/day (26). This value was derived from myelinated neurons in situ. Less precise information is available about the rate of regrowth of nonmyelinated adrenergic neurons (postganglionic nerves), the only studies being those relating to the reinnervation of the nictitating membrane and the surgically denervated heart (27, 28). Although these observations suggest a slower rate of regrowth of postganglionic nerves, even this could account for the re-
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establishment of the normal microanatomical pattern in the heart which we have studied following recovery from the failure state. The distances involved in this process are merely those between the preterminal nerves in the intermuscular septa and the terminal varicose elements within the muscle bundle, a distance probably not exceeding 100 to 200 μ. Regardless of whether regrowth of neurons or reconstitution of neuronal function is responsible for the present findings, it is clear that cardiac adrenergic neurotransmitter depletion is relatively rapidly reversible on recovery from heart failure.

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