Circulatory Responses to Electrical and Reflex Activation of the Nervous System after Cardiac Denervation

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ABSTRACT

Rigorous testing procedures assessed the extent of extrinsic cardiac denervation in three groups of animals subjected to cardiac extirpation and reimplantation (E&R), mediastinal neural ablation (MNA) or thoracic ganglionectomy (BSTG). After E&R, all dogs were completely denervated. Evidence for vagal reinnervation appeared as early as 26 days and for both vagal and sympathetic reinnervation in 74 days. After one, two and three years, the functional responses to reflex and electrical activation of the nervous system were similar to those in normal dogs. After MNA, only one animal of eight was totally denervated. Seven showed varying degrees of cardiac activation in response to diencephalic stimulation or stimulation of the cardiac sympathetic nerves. Thus MNA, though surgically feasible, cannot be assumed to be successful without rigorous testing. After BSTG, four of eight dogs showed complete separation of sympathetic cardiac outflow from the CNS; in all eight, moderate to large cardio-accelerator responses were elicited from the distal vago-sympathetic trunk after atropinization. There is little correlation between the over-all cardiac responses persisting in all operated animals and total cardiac catecholamine content, or between cardio-accelerator responses and right atrial catecholamine content.

ADDITIONAL KEY WORDS cardiac extirpation and reimplantation mediastinal neural ablation regeneration of extrinsic cardiac nerves cardiac autotransplantation diencephalic cardiovascular regulation vagosympathetic accelerator fibers cardiac denervation bilateral stellatectomy and thoracic ganglionectomy bilateral carotid occlusion cardiac catecholamines tests of anesthetized dogs and cats

A wide variety of extirpative procedures has been employed to denervate the heart. These neural ablations are designed to prevent neurally mediated alterations in cardiac activity during experimental procedures. Current denervation techniques include isolation and perfusion of the heart, sympathetic ganglionectomy and/or vagotomy, mediastinal neural ablation, and excision and reimplantation of the heart. In recent years, studies have been reported on cardiovascular function in dogs with chronic denervation.1-8 It is becoming increasingly clear that each of these denervation procedures offers certain advantages and imposes certain limitations.

In order to determine the extent of denervation more precisely, we have done a series of experiments designed to assess rigorously the degree of extrinsic cardiac denervation following three extirpation techniques. These experiments are the basis of this report.

METHODS

Experiments were performed on 17 adult mongrel dogs and 6 cats. All animals were anesthetized with phenycyclidine hydrochloride,* 2

* Sernyl, generously supplied by Parke, Davis and Company.
mg/kg intramuscularly, followed by intravenous α-chloralose in Carbowax (dogs: 80 to 100 mg/kg; cats: 40 to 60 mg/kg).

DENERVATION TECHNIQUES

1. Extirpation and Reimplantation of the Heart (E&R)

Seven dogs were studied three days to three years following extirpation and reimplantation of the heart (cardiac autotransplantation). This operation was performed with the aid of extracorporeal circulation. It involved division of the azygous vein, superior and inferior vena cavae, the pulmonary artery and the aorta. Division of the left atrial wall at the confluence of the pulmonary veins completed the separation of the heart from the body. The heart was restored to its original position by suture anastomosis.

2. Mediastinal Neural Ablation (MNA)

Six cats and two dogs were studied 8 to 72 days following mediastinal neural ablation. This procedure is based on the excision of all nervous tissue entering and leaving the heart. The heart and adjacent segments of great vessels were separated from their mediastinal attachments. The soft tissues containing the cardiac neural plexuses were excised, the pericardium was removed, and the blood vessels were stripped of their adventitial layers. This mediastinal neural ablation leaves the paravertebral ganglionated sympathetic trunks and the main vagal trunks intact. The denervation does not include other viscera or large vascular beds. All preganglionic fibers and postganglionic axons of extracardiac origin are presumably divided.

3. Bilateral Stellatectomy and Thoracic Gangliectomy (BSTG)

Eight dogs were studied 7 to 19 days following bilateral removal of the stellate ganglia and varying levels of the thoracic sympathetic trunk from T2 through T5. The thorax was opened and the third rib was removed on the right side. The sympathetic trunk at the levels indicated above, the stellate ganglia and the ansa subclavia were dissected and excised bilaterally. The caudal and cranial cervical sympathetic ganglia, and the vagus nerves were left intact.

By the techniques of E&R and MNA the objective is total extrinsic denervation of the heart; by BSTG, on the other hand, the aim is the separation of sympathetic outflow to the heart from the central nervous system, recognizing the limitation that it leaves intact postganglionic fibers in the caudal and cranial cervical sympathetic ganglia, and in other cardiac nerves.

TESTING PROCEDURES

In order to assess the completeness of denervation or the extent of persisting neural path-

ways to the heart, a series of rigorous experimental procedures was applied to all animals.

1. Carotid Sinus Reflex

Responses were measured during bilateral occlusion of the common carotid arteries (BCO) for periods of 30 to 60 seconds.

2. Afferent Nerve Stimulation

A. Stimulation of the central end of the cut sciatic nerve with bipolar electrodes at 4 to 8 volts, 2 msec and 50 cycles/sec.

B. Stimulation of the central end of the cut right and left vagosympathetic trunks in the neck with bipolar electrodes at 3 to 5 volts, 2 msec and 30 to 100 cycles/sec.

3. Stimulation of the Diencephalon

In the cat, the diencephalon was explored at stereotaxic plane A8,11 which is a section through the posterior hypothalamus. At this plane, electrodes were spaced at 1, 3, and 5 mm to each side of the midline. Stimulation at each of these points was done from the most ventral aspect of the brain stem to a level in the midthalamus (stereotaxic coordinates H — 6 to H + 3). In the dog, the brain stem was explored with an identical bank of electrodes at plane R15 or R18 from a level of H5 to H17.12 Identical bipolar concentric needle electrodes were used in all animals. Stimulation parameters were 2 to 6 volts, 2 msec and 100 cycles/sec.

4. Autonomic Nerve Stimulation

A. The distal ends of the cut right and left vagosympathetic trunks in the neck were stimulated with bipolar electrodes at 2 to 4 volts, 5 msec and 10 to 30 cycles/sec. Stimulations were performed before (to test for parasympathetic slowing) and after administration of atropine sulfate (0.5 to 1.0 mg/kg) to test for acceleration from excitation of cervical sympathetic fibers.

B. In the E&R and MNA animals, the stellate ganglion on each side was stimulated at 2 to 4 volts, 5 msec and 10 cycles/sec. In the BSTG animals, depending on the specific operation performed, any remaining thoracic segments of the sympathetic trunks between T2 and T5 were stimulated at the same parameters used for the stellate ganglia.

MEASUREMENT TECHNIQUES AND INSTRUMENTATION

Blood pressure was recorded from a catheter introduced into the femoral artery and advanced up into the thoracic aorta. Pressure was detected by a Statham P23Db transducer, amplified with a Honeywell model 130 carrier amplifier, and recorded photographically in a Midwest model 591 optical oscillograph. The signal from the carrier amplifier was also sent through a filter circuit...
to another galvanometer of equal sensitivity and zero base line. This provided a continuous dual recording of pulsatile and mean aortic blood pressures superimposed on one another. Right ventricular contractile force was measured with a strain gauge arch sutured to the right ventricle. The strain gauge signal was amplified and recorded in a manner identical with the pressure recordings. Electrocardiograms were recorded using Argonaut model LR042 differential preamplifiers. Heart rate was recorded continuously from an integrating cardiotachometer. Both heart rate and mean blood pressure were monitored continuously by direct readout on Digitec digital voltmeters, and all parameters of measurement were monitored visually on a multichannel Rycom oscilloscope. Stimulation pulses were derived from a Grass model S4 stimulator with an isolation unit, and stimulation parameters were monitored on a Tektronix model 502 dual beam oscilloscope.

Prior to sacrificing the animals, lesions were placed in the brain at known stereotaxic coordinates using a Grass RF lesion maker delivering 100 milliamperes for 30 seconds. The brains were removed and fixed in formalin for subsequent histological examination of serial sections. The heart was removed and rapidly divided into five portions (right and left atria, left ventricle, free wall of right ventricle and septum). These portions were immediately placed into liquid nitrogen and subsequently analyzed for catecholamines using the trihydroxyindole method of Crout et al. Injection of small doses of epinephrine or noradrenaline (0.25 μg/kg) from a catheter in the inferior vena cava at the level of the diaphragm produced similar responses after a latency of 8 to 10 seconds.

The magnitude of the pressor (vasoconstrictor) response to carotid occlusion or diencephalic stimulation was relatively small, amounting usually to 30 to 50 mm Hg. The greatest responses in these animals were elicited by stimulation of the central end of the cut cervical vagosympathetic trunk. The initial response was a large rise in mean aortic pressure, with no significant change in heart rate or right ventricular contractile force, followed by a secondary cardiac response after a latency of 14 to 18 seconds. The secondary response again appeared to be due to reflex activation of adrenal medullary secretion. Figure 1 is an example of this kind of response elicited by stimulation of the central end of the cut left cervical vagosympathetic trunk.

Two dogs were studied 26 and 74 days after autotransplantation of the heart. The data demonstrated the possibility of surprisingly early reinnervation of the heart. Surgical removal of the heart per se accomplished an unequivocal separation of all cardiac nerves from the central nervous system. Reimplantation establishes a series of suture lines which presumably offer a considerable barrier to the re-establishment of neural outflow to the heart. Despite these facts, dog E&R 21 (26 days postoperative) showed clear evidence of reinnervation of the heart by a few fibers from the right vagosympathetic trunk. Supramaximal stimulation of the distal end of the cut right vagosympathetic trunk in the neck caused a consistent and reproducible slowing.
Delayed cardiac responses of adrenal origin following stimulation of central end of cut left vagosympathetic trunk in an E&R dog 13 days after autotransplantation of the heart. Traces from top to bottom: right ventricular force, heart rate, superimposed mean and pulsatile aortic pressures, event marker, time base at one-second intervals.

of heart rate from 170/min to 144/min. Administration of atropine abolished this response. Stimulation of the left vagus had no effect on heart rate. Responses to the other testing procedures confirmed the fact of total extrinsic denervation of the sympathetic supply to the heart. Dog E&R 37, studied 74 days postoperative, showed evidence of moderate reinnervation of the heart by both sympathetic and parasympathetic fibers. Stimulation of either distal vagosympathetic trunk resulted in pronounced bradycardia before administration of atropine. After atropine, cardio-acceleration of 40 beats/min was elicited by electrical stimulation of the right vagosympathetic trunk, but no effect was obtained from the left. Moderate to large cardiac acceleration and augmentation were observed when the stellate ganglia or diencephalon was stimulated. A small cardiac effect occurred in response to carotid artery occlusion. No responses were elicited by afferent nerve stimulation.

Three dogs studied one, two and three years after autotransplantation showed evidence of extensive reinnervation of the heart. Exploration of the diencephalon revealed extensive areas, stimulation of which resulted in massive pressor responses with large increases in heart rate and myocardial contractility. Similarly marked cardiac activation was produced by stimulation of the sciatic nerve and stellate ganglia, and by stimulation of the distal cervical vagosympathetic trunk after atropine. In all three animals reinnervation of the heart by efferent vagal fibers was demonstrated by elicitation of cardiac arrest when the distal cervical vagosympathetic trunks were stimulated before atropine. The extent of functional reinnervation is indicated in figures 2 and 3. Figure 2 illustrates the response to stimulation in the posterior hypothalamus before vagotomy in a dog three years after E&R. A great activation of nervous outflow to the heart occurred after a brief latency. A similar response was observed after vagotomy, except that the heart rate continued to increase...
after cessation of stimulation. Return to control level was prolonged after vagotomy. Figure 3 shows the response in the same animal to stimulation of the central end of the cut left vagosympathetic trunk, indicating again a rapid and large cardiac activation.

MEDASTINAL NEURAL ABATION SERIES

Of the six cats and two dogs in which mediastinal neural ablation was performed, only one cat (MNA 31) showed complete extrinsic cardiac denervation as tested by our procedures. Another cat (MNA 7) exhibited almost complete denervation, with the exception of a few persisting fiber pathways which were demonstrated by small cardiac responses when the posterior hypothalamus

**FIGURE 2**

Cardiac responses to hypothalamic stimulation in an E&R dog three years after autotransplantation of the heart. All traces as in figure 1.

**FIGURE 3**

Cardiac responses to stimulation of central end of cut left cervical vagosympathetic trunk in an E&R dog three years after autotransplantation of the heart. All traces as in figure 1.
and stellate ganglia were stimulated. The other six animals showed varying degrees of persisting cardiac responses ranging from moderate to large. Of the eight animals in this series, seven showed varying degrees of positive sympathetic cardiac responses to diencephalic stimulation. Figure 4 is a composite of two responses in one of the dogs in this series. Part A illustrates the response to stimulation in the posterior hypothalamus at stereotaxic coordinates R18, RL3, and H8, and indicates a massive cardio-acceleration (HR increased from 110 to 200/min), with minor changes in systolic and diastolic pressures. Part B shows the response recorded when another electrode 2 mm medial to that indicated above was stimulated at the same parameters (4 volts, 2 msec and 100 cycles/sec). In this case a still greater cardio-acceleration occurred, as well as a significant rise in systolic and diastolic pressures. Four of the eight MNA animals showed cardiac responses to bilateral occlusion of the carotid arteries, two to afferent nerve stimulation, seven to thoracic sympathetic trunk stimulation and five to stimulation of the distal ends of the cut cervical vagosympathetic trunk after atropinization. Three animals exhibited cardiac slowing or arrest when the distal vagosympathetic trunk was stimulated before the administration of atropine. A striking finding in both dogs in this series (MNA13 and MNA6) was the fact that mediastinal neural ablation successfully removed all extrinsic efferent vagal innervation, but left intact significant accelerator responses from fibers in the cervical vagosympathetic trunks. Stimulation of the right distal cut end of this nerve in each of the two dogs produced cardiac acceleration from 150/min to 218/min, and from 142/min to 220/min, respectively. Figure 5 illustrates the persistence of both vagal and sympathetic responses to stimulation of the distal vagosympathetic trunk in a cat. Parts A and B show the response to stimulation of the cut ends of right and left vagosympathetic trunks prior to administration of atropine, with resultant bradycardia or cardiac arrest. Parts C and D illustrate a repetition of the same procedures after intravenous injection of 0.5 mg/kg of atropine sulfate. Stimulation now produced a moderate acceleration from the left side (HR increased from 125 to 140/min), and a large acceleration from the right side (HR increased from 120 to 190/min).

Despite the presence of large functional responses, it is not possible to assess the number of persisting fibers involved. In neither case was there evidence of augmented myocardial contractility in the right ventricle. It is possible therefore that a few fibers terminating in or near pacemaker tissue could exert this large functional effect.

**BILATERAL STELLATECTOMY AND THORACIC GANGLIONECTOMY SERIES**

Of the eight dogs studied in this series, four showed complete decentralization of
CIRCULATORY RESPONSES AFTER CARDIAC DENERVATION

sympathetic cardiac outflow from the central nervous system. In the remaining four animals, small to moderate sympathetic cardiac responses were obtained with stimulation in the diencephalon. Three animals gave positive cardiac responses to carotid artery occlusion. On the other hand, none of the animals demonstrated cardiac activation by afferent nerve stimulation, and only one animal gave a small cardiac response to stimulation of the remaining portion of the thoracic sympathetic trunk. In this animal, which had been sympathectomized bilaterally through T3, the small response occurred when the T5 level was stimulated.

As would be expected from the nature of the denervation procedure in this series, all eight animals subjected to BSTG showed cardiac arrest when the distal cervical vagosympathetic trunk was stimulated before atropine was administered. Similarly all animals exhibited large cardioacceleratory responses when the same stimulation was performed after atropine, the largest response being an increase in heart rate from 100/min to 250/min. Since the denervation procedure does not interrupt postganglionic neurons in this nerve, it is reasonable to assume that the observed responses in these animals are representative of the normal cardiac nerves present in this combined nerve trunk. As with animals in the other two series in which persisting innervation remained in this pathway to the heart, stimulation of the right cervical vagosympathetic trunk after atropinization produced greater cardioacceleration than the left nerve trunk. The average increase in heart rate for the eight BSTG animals was 51/min from the right and 24/min from the left vagosympathetic trunk. This difference is statistically significant (P<0.01). Augmentation of right ventricular contractility was
again small (less than 15% increase above control) or absent.

Table 1 is a summary of all data for the three series of animals. Responses have been graded on a +1 to +4 basis, the latter being equivalent to the response obtained in normal dogs. No response is indicated by a 0. The rating of certain responses, such as BCO, is relatively simple, a +1 being approximately 25% of the response obtained in normal dogs. In other cases the rating is more complex, since it must assess the contribution from both right and left nerves of stellate ganglia, or the over-all responses to stimulation of many points in the diencephalon. These assessments of necessity include a certain degree of subjective evaluation. The base line reference of responses in normal animals provides a reasonable standard for comparison, and one of the authors (CNP) has recorded data on over 200 animals which provide this reference standard.

**CATECHOLAMINE LEVELS VS. CARDIAC RESPONSES**

Catecholamine levels of the five portions of the heart were obtained from 21 of the 23 animals used in these experiments. Correlation of catecholamine content with persisting cardiac response was attempted in two ways:

1. Total heart catecholamine concentration was compared with over-all cardiac responses in each animal. Over-all cardiac response was graded as +1 to +4, the latter figure being based on the responses determined in normal dogs.

2. In similar fashion right atrial catecholamine concentration was compared with the cardio-accelerator response of each animal, again rated on a +1 to a +4 basis.

### Table 1

**Summary of Data on Cardiac Responses in All Animals Following Various Denervation Procedures**

<table>
<thead>
<tr>
<th>Type</th>
<th>Days post-operative</th>
<th>Symp. cardiac responses</th>
<th>Symp. trunk stim.</th>
<th>Distal vagus parasymp.</th>
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<td>++ 0 0 0</td>
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</tr>
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<td>++ +</td>
</tr>
<tr>
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<td>++ +</td>
</tr>
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<td>0 0</td>
</tr>
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</tr>
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E&R: extirpation and reimplantation.
MNA: mediastinal neural ablation.
BSTC: bilateral stellatectomy and thoracic gangliectomy.
BCO: bilateral carotid occlusion.
*Cats; all others were dogs.

Circulation Research, Vol. XIX, July 1966
Figure 6 illustrates the results of these comparisons. The dashed line in each part of the figure was constructed by drawing a line from the zero intercepts to a point determined by a normal response (+4) and the corresponding catecholamine contents for total heart and right atrium in normal dogs. It can be seen that there is little consistent relationship in either case. On the left side of the figure it is readily apparent that extremely low catecholamine concentrations were found in animals exhibiting moderate to large cardiac responses. For example, total heart catecholamine concentrations were 0.03 μg/g wet wt in animal E&R 9, and 0.06 μg/g wet wt in animal E&R 22. Animal E&R 9 was studied nine days postoperatively and showed total extrinsic cardiac denervation. Animal E&R 22, which was studied two years after autotransplantation of the heart, demonstrated extensive reinnervation with massive cardiac responses in both rate and contractility.

It is also striking that catecholamine levels were so low in the three E&R animals which had undergone extensive reinnervation, as judged by return of functional responses. This same lack of correlation holds true in the MNA and BSTG animals. In the MNA group, moderate cardiac responses were demonstrated in animals having total heart catecholamine concentrations ranging from 0 to 0.60 μg/g wet wt. All of the BSTG animals had greater total heart catecholamine levels than animals in the other two series. This finding may be explained in part by the persistence of intact sympathetic postganglionic fibers with cell bodies in the cranial and caudal cervical sympathetic ganglia, which are not disturbed by the operative procedures in this group of animals. Despite this finding, however, removal of the stellate ganglia and varying levels of the thoracic sympathetic trunks successfully separated the heart from central connections in 50% of the animals. In spite of this loss of ability to respond to CNS stimulation or reflex activation, catecholamine concentrations in some animals were normal. For example, animal BSTG 20, which showed no cardiac responses to these testing procedures, had an average atrial catecholamine concentration of 2.06 μg/g wet wt, and an average ventricular level of 0.57 μg/g wet wt.
Table 2

Summary of Catecholamine Data for All Animals

<table>
<thead>
<tr>
<th>Type</th>
<th>Right atrium</th>
<th>Left atrium</th>
<th>Right ventr.</th>
<th>Left ventr.</th>
<th>Ventr.</th>
<th>Total heart</th>
<th>Over-all</th>
<th>Accelerator</th>
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<td>Normal dogs</td>
<td>2.64 ± 1.2</td>
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<td>BSTG14</td>
<td>0.19</td>
<td>0.59</td>
<td>1.69</td>
<td>0.94</td>
<td>0.38</td>
<td>0.94</td>
<td>0</td>
<td>+</td>
</tr>
<tr>
<td>BSTG12</td>
<td>2.43</td>
<td>1.09</td>
<td>0.81</td>
<td>0.44</td>
<td>0.46</td>
<td>0.66</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>BSTG17</td>
<td>0.56</td>
<td>0.63</td>
<td>0.48</td>
<td>0.33</td>
<td>0.33</td>
<td>0.38</td>
<td>0</td>
<td>++</td>
</tr>
<tr>
<td>BSTG20</td>
<td>0.97</td>
<td>0.51</td>
<td>0.81</td>
<td>0.65</td>
<td>0.69</td>
<td>0.71</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

*Note: CATS.

Table 2 is a summary of the catecholamine data in all animals.

Discussion

These experiments serve to define certain limits in the attainment of extrinsic cardiac denervation by these different techniques. They also raise significant questions concerning the methods by which the extent of denervation can properly be assessed. The extensive testing procedures used in these experiments unequivocally demonstrate neural connections to the heart, and indicate that the procedure of cardiac autotransplantation (E&R) achieves total extrinsic cardiac denervation. This is not surprising because surgical extirpation of necessity provides for the sectioning of any and all nerve fibers entering the heart, regardless of their individual pathways within the thorax. On the other hand, the observation that reinnervation may occur within 26 days after autotransplantation was unforeseen. It had been reported by Willman et al.15 in a series of 15 dogs subjected to E&R that reinnervation did not occur before 10 months after operation. The presence or absence of reinnervation was determined by the responses to stimulation of the cervical vagosympathetic trunks and the stellate ganglia in these dogs under sodium thiopental anesthesia. In 5 of the dogs cardiovascular responses to administration of tyramine and norepinephrine were also used to evaluate whether or not reinnervation had occurred. In the E&R series reported in this paper, only the two dogs studied at 3 and 13 days postoperatively were found totally free of extrinsic cardiac innervation. Dog E&R 21, studied 26 days after operation, showed evidence of a small but significant vagal slowing of the heart, which can be explained only by

Circulation Research, Vol. XIX, July 1966
reinnervation. Dog E&R 37, studied 74 days after operation, showed evidence of significant functional reinnervation by both sympathetic and parasympathetic fibers to the heart (table 1).

The only significant difference between methods used in these two sets of experiments was the anesthetic agent employed. It is well known that barbiturates depress central nervous transmission of cardiovascular reflexes in small doses, and that large doses (above 19 mg/kg) have direct depressant effects on the myocardium. It has also been shown that vagal inhibition of heart rate is depressed by barbiturates. It is equally true, on the other hand, that positive chronotropic and inotropic responses to stellate ganglion stimulation can be elicited in animals fully anesthetized with barbiturates. The possibility exists that these anesthetics have more profound effects when a relatively few fiber connections to the heart are involved, as would presumably be the case in the early reinnervation period following a denervation procedure. We have noted, for example, that a small dose of sodium pentobarbital (5 mg/kg), which has no significant effect on cardiac responses to stellate ganglion stimulation, does depress the accelerator response to stimulation of the right vagosympathetic trunk in the normal dog. This may be related to the fact that relatively few of the total number of sympathetice accelerator fibers to the heart are present in this nerve trunk. It has also been shown (Peiss and Randall, unpublished data) that small doses of pentobarbital successfully block vagal inhibition of heart rate in experiments where the number of vagal fibers being stimulated is small. Despite the return of cardiac responses to essentially normal magnitude, the catecholamine content of the various chambers of the heart remained below that of normal dogs. These observations suggest the possibility of a significant functional and transmitter reserve in the heart's innervation. It is our impression, based on examination of data from animals in all three series, that a relatively few persisting fibers can have large functional effects. Striking support of this impression is found in the BSTG animals, in which the primary sympathetic outflow to the heart has been sectioned. Despite this, extremely large cardio-acceleration can be obtained when only the accelerator pathway in the cervical vagosympathetic trunk is stimulated.

The recent studies on cardiovascular responses of animals with chronic cardiac denervation by means of the mediastinal neural ablation technique have assessed the completeness of denervation in acute experiments, following the procedure of Cooper et al., and using one or another barbiturate as an anesthetic agent. The experiments reported in this paper emphasize very sharply the following conclusion: it is possible to accomplish total extrinsic cardiac denervation by means of mediastinal neural ablation, but this requires extreme diligence in surgical technique in order to expose and excise all potential neural pathways to the heart. Moreover, rigorous testing procedures must be adopted to assess fully the extent of denervation in any given animal. In our experiments only one animal in a series of eight showed evidence of total extrinsic cardiac denervation. The failure to demonstrate grossly any remaining pathways in postmortem examination again supports the impression that the significant functional residue in these animals might be due to a relatively few persisting nerve fibers to the heart. In both dogs studied in the MNA series, the heart was successfully denervated with respect to vagal inhibitory fibers. However, in each case there persisted moderate to large cardio-accelerator responses when the distal end of
the cut cervical vagosympathetic trunk was stimulated. The possibility exists that these responses may be mediated by nerve fibers coursing intramurally within the large blood vessels in the thorax, thus escaping interruption despite surgical stripping of the adventitia. If this were true, doubt would exist concerning the predictability of success for mediastinal neural ablation even when the operation was performed with extreme diligence. This point is extremely important in assessing cardiac responses to exercise or other stresses in the "chronically denervated state." Nerves have been observed in the adventitia of the aorta by electron microscopy although they have not been detected in the media.

Data from animals subjected to cardiac extirpation and reimplantation indicate the possibility of reinnervation within 26 days, despite the fact that regenerating fibers must of necessity cross suture lines. The period of reinnervation in MNA animals is not known. Determination of reinnervation time will require a series of MNA animals with proven total extrinsic cardiac denervation that can be followed sequentially. Since the animals in our MNA series that showed some evidence of persisting innervation were studied from 8 to 72 days after operation, it is possible that the functional responses observed in some of these animals could be the result of reinnervation rather than incomplete denervation. Autonomic regeneration is considered to proceed at a rate of approximately 1 mm/day. In the cat, therefore, one could estimate about four weeks as the minimum time for possible reinnervation of the heart after mediastinal neural ablation. Although our experiments give no information concerning the physiological activation of the heart in chronically denervated, unanesthetized animals, they do raise serious doubts as to the validity of assuming total extrinsic cardiac denervation following mediastinal neural ablation. It is our opinion that far more rigorous testing procedures are required to demonstrate totality of denervation than those usually employed. Most importantly, our experiments indicate that the assessment of denervation in acute animals requires some anesthesia other than the commonly used barbiturates.

Bilateral excision of the stellate ganglia with or without more caudal portions of the thoracic sympathetic trunk through the T5 level (BSTG) is very effective in accomplishing functional separation of the sympathetic supply to the heart from the central nervous system. Four of the eight animals in this series showed total functional separation as tested by stimulation of the diencephalon or of the afferent limbs of cardiovascular reflexes. Since the entire cervical sympathetic trunk is intact in these animals, it appears that the great preponderance of preganglionic sympathetic cardiac outflow must pass through the stellate ganglia. It is improbable that reinnervation has occurred in these dogs since the maximum time at which they were studied postoperatively was 19 days. In all eight of these animals, however, large cardio-accelerator responses were obtained when the distal cervical vagosympathetic trunk was stimulated after administration of atropine. The accelerator responses were generally much greater from the right vagosympathetic trunk. Two possible explanations can account for these responses:

1. Because stimulation was delivered between the cranial and the caudal cervical sympathetic ganglia, these accelerator responses could be due to the presence of intact sympathetic postganglionic fibers, whose cell bodies are in the cranial cervical sympathetic ganglion. Failure to activate these fibers by reflexes or CNS stimulation would imply therefore that the preganglionic connections to these neurons had been severed by the bilateral stellate gangliectomy.

2. The accelerator responses could be due also to the presence of accelerator fibers in the vagus nerve, as reported by many workers. Kabat in particular has demonstrated the existence of these fibers in experiments that appear to be conclusive. Using dogs under chloralose anesthesia, he reported that stimulation of decentralized vagal rootlets in the cranium resulted in significant car-
dio-acceleration after administration of atro-
pine and section of the spinal cord at C2. Kabat also reported that these accelerator 
fibers could not be activated by the usual cardiovascular reflexes.

The small responses to reflex and CNS activa-
tion in four of the eight BSTG animals cannot be accounted for at present by definite 
knowledge of persisting nerve fibers in any particular pathway. In one of these four ani-
mals a small effect was obtained by stimu-
lation of the sympathetic trunk at T5. In 
other animals, however, total separation of 
the heart from central sympathetic responses 
was obtained with removal of the stellate 
ganglia only. The possibility does exist, how-
ever, that thoracic outflows below T5 could 
account for the persisting responses in some 
animals. It is equally possible that direct CNS 
or reflex activation of the vagal accelerator 
outflow is involved. At present little is known 
about the location or central connections of 
the accelerator fibers that have been shown 
to exit from the brain stem through vagal 
rootlets. Aberrant embryologic migration in-
to the vagus nerve might derive from medul-
ary, hypothalamic and cortical fibers which 
normally traverse the brain stem, descend into 
the spinal cord and terminate on pregang-
lionic sympathetic cells. If these vagal accel-
erator fibers do represent a random aberrant 
migration from various levels of the central 
nervous system that have a role in cardio-
vascular function, it might explain the dif-

culty of activating them reflexly or by direct 
CNS stimulation, since they might be derived 

from widely separated parts of the brain. 

Catecholamine content of the several parts 
of the heart following these various methods 
of cardiac denervation appears to bear little 

relationship to the extent of persisting func-
tional response. Shortly after autotransplanta-
tion of the heart, catecholamine levels are 

close to zero and the animals show total ex-
trisic cardiac denervation. Animal E&R 8, 

which also showed total extrinsic denervation, 
did have significant but lower than normal 
levels of catecholamines. This was most 
probably the result of insufficient time for 
depletion of catecholamines, since the animal 
was studied only three days after operation. 

On the other hand, E&R animals which 

had undergone extensive reinnervation never 

re-established catecholamine levels compar-
able to those of the normal, nonoperated ani-

mals shown in table 2. There is no way at 

present to determine the actual density of 

postganglionic terminations that have been 

re-established on the heart as compared with 

that in the normal dog. We can only assess 

the functional responses that can be derived 

from the re-established neural connections, 

and these are essentially similar in magni-

tude to those of the normal dog. If there is a 

significant reserve in the innervation of the 

heart, it would be a factor in explaining the 

lack of correlation between functional re-

sponses and catecholamine content. This pos-

sibility is supported by the data from MNA 

animals. On the basis of the extremely low 

catecholamines, surprisingly large functional 

responses were obtained in most of these ani-

mals. The possibility remains that these re-

sponses are mediated by a very small number 

of intact neural connections. It is significant 

that Gaffney et al. have shown significant de-

pletion in myocardial catecholamine content 

by reserpine, associated with apparently nor-

mal accelerator responses to sympathetic 

nerve stimulation.29

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Circ Res. 1966;19:153-166
doi: 10.1161/01.RES.19.1.153

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