Original articles

Alterations of cardiac innervation in evolving myocardial infarction and transplanted hearts. An anatomo-clinical reappraisal

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Key words:
Autonomic neuropathy; Heart transplantation; Myocardial infarction.

Background. **Debate regarding the alterations of the cardiac innervation in an evolving myocardial infarction and transplanted hearts is still raging and most studies are based on radionuclide uptake of neurotransmitters or on the evaluation of the cardiorespiratory reflex.**

Methods. **The present investigation, upon human autoptic specimens of 57 infarcts and 8 cardiac transplants, was carried out with traditional neuropathology and modern molecular biology techniques. The specimens were selected for the identification of neurons, nerve fibers and their sheaths.**

Results. **First of all, these techniques confirmed the gross difference in the vulnerability of infarcted myocytes if compared with the local innervation, the metabolism of which is infinitely less oxygen-dependent than that of working myocardium (approximate quantitation below). Delicate technicalities of the traditional silver impregnation for nerves usually yield a large incidence of artifacts. Thereby, only perfect results (20% of cases), corroborated by parallel nerve sheath immunostaining (70% of cases), were retained and documented herein. In the meantime, acidosis and free radicals increase, while catabolite accumulation supervenes. These three factors threaten myocardial viability. Thereby, nervelets can be seen to survive the hyperacute phase of ischemia, but may be in part damaged by the successive granulocytic-macrophage inflammation enzyme lysis of the infarcted muscle. The delayed and incomplete anatomical neural damage is confirmed by the observation of preserved nerve sheaths and neural filaments surviving in postinfarction scars, almost devoid of myocardiocytes.**

Conclusions. **The rich sympatho-vagal cardiac network might further provide alternative bypasses for post-infarct reinnervation. The functional implications of this process remain unclear.**

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Methods

From 65 human autoptic hearts (57 infarcted and 8 transplanted), fragments were excised and fixed in buffered formalin for neuropathological evaluation. In myocardial infarctions (38 males and 19 females, mean age 65 years) the episode of severe ischemic injury was timed at between 2 hours and 22 months. In these cases, the sinoatrial node and the conduction system, together with the entire junctional and septal conduction systems, were sliced in series (every 200 μ), together with free walls samples taken from the infarcted areas. The timing of severe acute ischemia/infarction is approximate, since it refers to the unreliable coincidental synchrony with the clinical manifestations[1], without any information about the ever-important timing and type of emergency treatment.

The specimens were either frozen or paraffin embedded, and the sections stained with the following methods for neural structures: classic silver impregnation and variants[2] altogether ensuring the demonstration of axons, dendrites and neuronal bodies. Anyone familiar with the inherent technicalities of these procedures is aware of their high incidence of artifacts, so that only perfectly silver impregnated specimens (from about 20% of cases) were illustrated.

Adjacent sections were examined by means of monoclonal antibody (Dako) staining for neurofilaments, with Dako Schwann cells S-100, and by means of the histochemical Kluver-Barrera method for the myelin sheaths (its Cresyl-violet-affinity also permitted the staining of the cytoplasmic tigroid substance).

Routine controls were carried out using the trichrome Heidenhain (Azan) and/or hematoxylin-eosin techniques. As may be deduced, any functional neurotransmitter staining has been left aside, so as not to

interfere with the restricted aim of the research (Figs. 1-6).

The herein documented evidence of nerves, neuronal appendages and sheaths refers to 70% of the infarctions, and to all the cases of transplanted hearts. No commitment has been made about the debated nature of Yamauchi's[3] neurotransmitters.

Basic queries concerning the type and degree of neural injuries ranging between structural destruction and milder lesions entailing lesser functional impairments still stand wide open.

In heart transplants (surgery performed from 2 days to 28 months previously) histology was focused, as rarely done, on either side of the atrial scar, in the region of both the recipient's and donor's (Figs. 5 and 6) sinoatrial node areas. Cases with transplant reject features, if severe and extensive, were discarded.

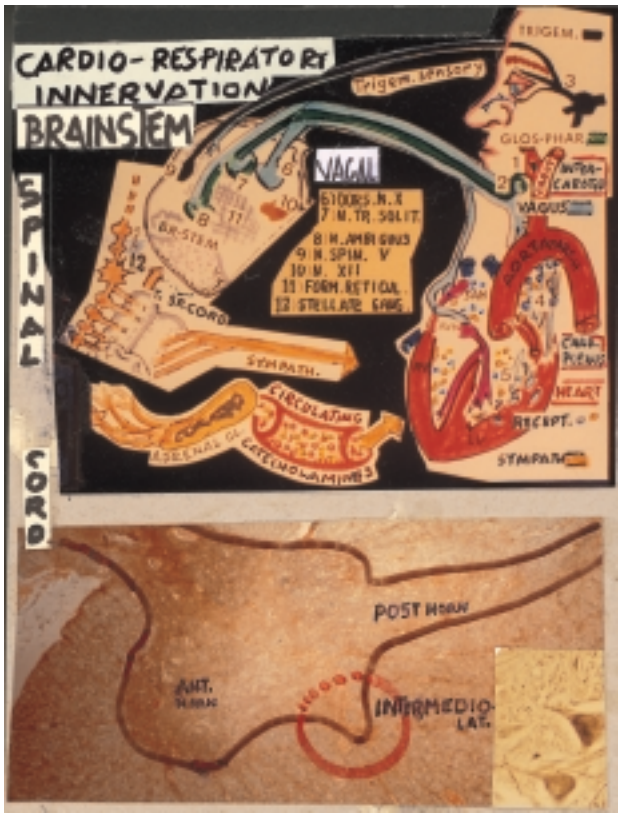

Figure 1. *Cardiorespiratory innervation. Vagal reflexogenic arousal, centers in the brain stem; sympathetic centers in the spinal cord T1-T4 internal mid-lateral Horn's neurons (Bielschowsky 10×, 800×).*

Results

As a premise to the results' interpretation, the author ought to better point out the supervened novel outlook of cardioneuropathology.

Indeed, while for years specialized anatomists and physiologists struggled with the intriguing complexities of the heart's innervation, recently a clever, dedicated researcher in clinical cardiology[4], so oversimpli-

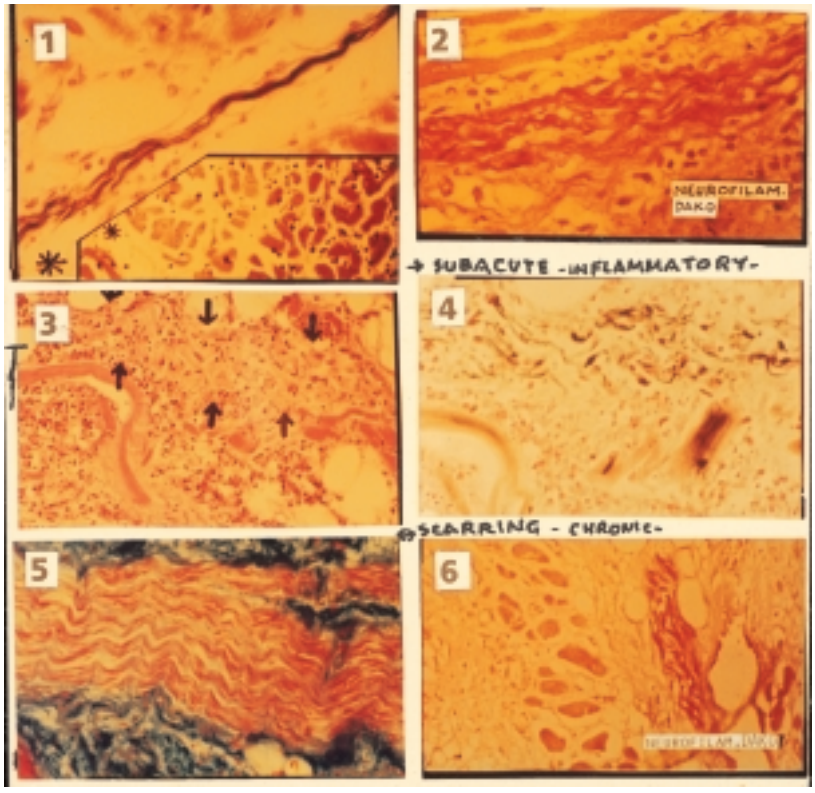

Figure 2. *Neuromyocardial injury in evolving infarct stages (1 and 4 silver impregnation 100×, 2 and 6 Dako-immuno 200×, 3 and 5 hematoxylin-eosin Azan 300×).*

fied the open queries as to localize into only three of the commonplace subepicardial "fat pad-plexuses", the very sites of the crucial autonomic nervous input to the heart[4,5]. The quoted author, however, overlooked the traditional as well as newer data of cardioneurology fundamentals in light- and electron-microscopy study[3,6-12] which indicated that both adrenergic and cholinergic fibers from the extracardiac vagi anastomize in the intrinsic heart plexus and may even run along individual sheaths of nervelets, throughout the intermingled sympathovagal cardiac supply[2,13]. The innovators, forgetting the newer data in cardioneurology[8,9], unreservedly adopted the novel techniques in radiolabeled neurotransmitter imaging[3,7-12], semantically set in between pathophysiology and structural pathology, and not helped by comparative anatomy, even in view of the fact that gross discrepancies do exist between mammals and humans[2,6,7,13,14].

The infarct's "tragedy" starts within seconds of a major coronary artery occlusion with the shift of the cardiac metabolism from aerobic to anaerobic, entailing a dramatic fall and slowdown of the vital myocardial oxygenation (approximate quantitation below). Thereupon, the chronology and severity of the cardiac tissues' injuries take different courses depending on the respective degrees of oxygen dependence, with the absolute prevalence and precocity of myocyte damage over that of the intrinsic innervation, subject to the unpredictable infarct injury[1,15].

Indeed, besides the debated fact that cardiac nerves are partially independent of the local coronary circulation[15], they also benefit from an axonal metabolic supply from the neuronal cells, often external to the ischemia, which run down along the axon to supply the terminals with neurotransmitter metabolites[16].

The most reliable conclusion is that drawn by Randall-Ardell in Zipes and Jalife's textbook[4] itself; indeed these authors stress "... the precise demonstration of the sympathetic and parasympathetic supply to the human heart remains conjectural".

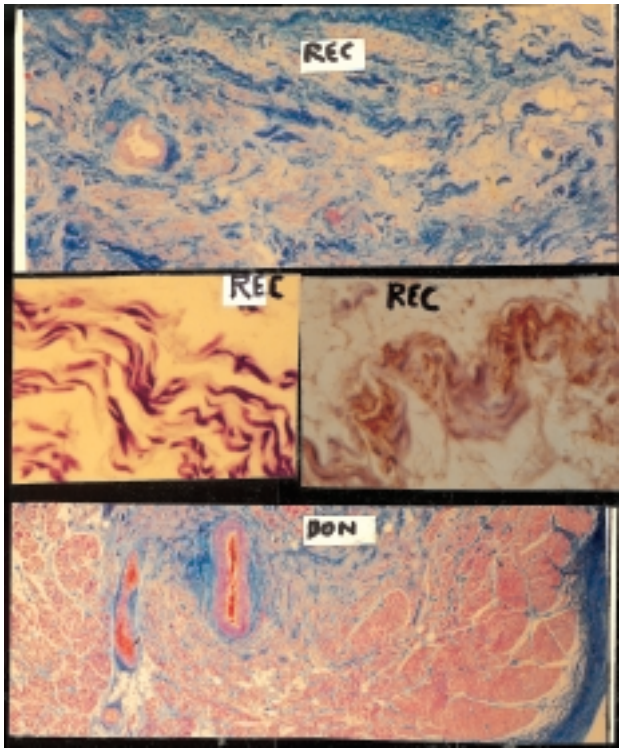

Figure 3. *Heart transplant surgery and nerves. Demyelinated receptor nerve and Schwann tunnel.*

Be this as it may, contradictory data from the literature[13,14] suggest that the infarct's sympathetic excitation with the reciprocal vagal imbalance depends particularly upon the anterior versus inferior ischemic involvement, as Malliani's spectral analysis seemed to demonstrate[11] and as the present research confirmed for the heart as a whole.

As a matter of fact, in hyperacute and acute infarcts (1 to 3 hours) the nervelets in the severely ischemic area

Figure 4. *Transplanted heart. Twin sinoatrial nodes and evidence of nerve demyelination in the recipient (Weigert Dako S-100, 500×).*

were free from axonal disruptions which, on the other hand, were seen to supervene in a successive subacute stage of the infarction injury (Fig. 2), approximately 3 to 8 hours following the attack, and seemed to closely follow the increasing polymorphonuclear-macrophage infiltration of the infarcted muscle participating in the lytic removal of ischemia-killed (or injured) heart muscle during the "transitional" stage of initial healing. The nervelets intrinsic to the infarcted area showed variable images of involvement, both in extension and severity, in so far as some nerve networks could have escaped the actual interruption of both the sheaths and axons that further present in the midst of thick myocardial scars (Fig. 3).

The fundamental discrepancies between the infarct's myocardial "catastrophe" and the intrinsic neural damage are self-evident, and do not require further comment. Thereupon the ever-important question about postinfarction reinnervation arises[3,5-13,16-20].

First of all, no histological evidence of attempted regeneration (such as Schwann-cell tubular hyperplasia) has ever been seen. The evidence of the partial survival of the nervelets within postinfarction scars, renders the proposal that infarcted hearts might undergo transplant-like cholinergic deafferentation unlikely[7], since the nerves are not totally severed from the central reflexogenic arousal. Moreover, with regard to postinfarction, the unsatisfactorily explained question of reinnervation did not take into sufficient consideration the abundant alternative routes supplied by the potential physiologic bypasses throughout the rich interlacing of the heart's sympatho-vagal intrinsic network[3,8-10].

Histological evidence of nerve regeneration is totally lacking whereas, as said, segments of nervelets and sheaths surviving the infarct are not rare, even within muscle-deprived thick scars.

Be this as it may, in the supposedly "denervated" postinfarct areas recovery was suggested to occur within 7 weeks or more[11], despite the lack of any histological harbinger.

Last but not least, the present results cast substantial doubts on Zipes and Jalife's 1990 textbook reiterated emphasis[17] regarding the interpretation of experiments with combined ^{123}I-meta-iodobenzylguanidine (MIBG) neurotransmitter images from epicardial Phenol brushing (carried out in Zipes Indiana University Laboratory[4]), a procedure allegedly demonstrating the adrenergic cardiac denervation and the contemporary disrup-

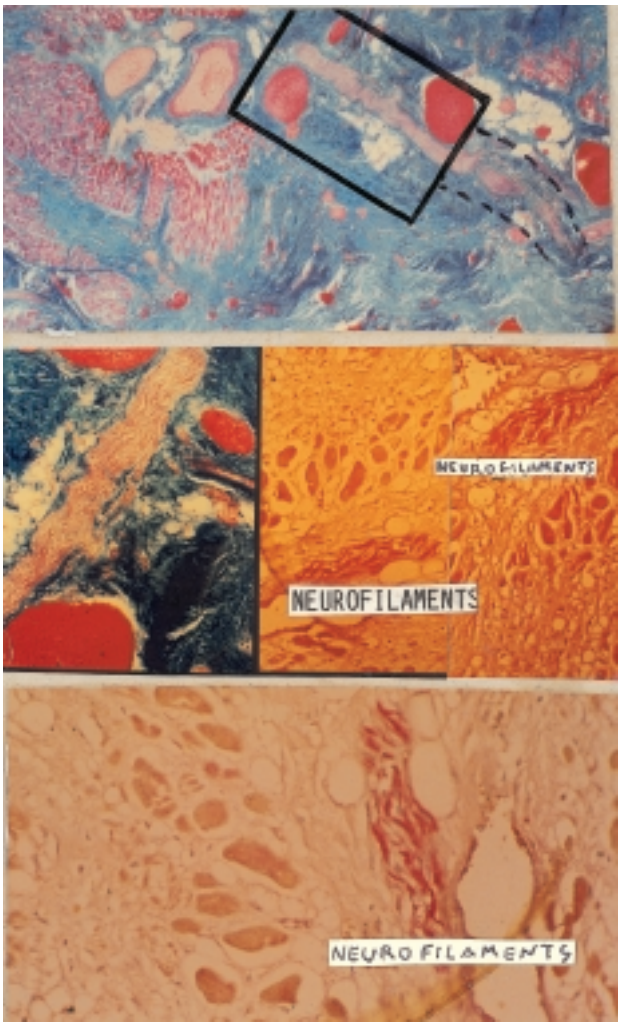

Figure 5. *Healed myocardial infarction with surviving nerves within scars (Azan and Dako neurofilaments 300×).*

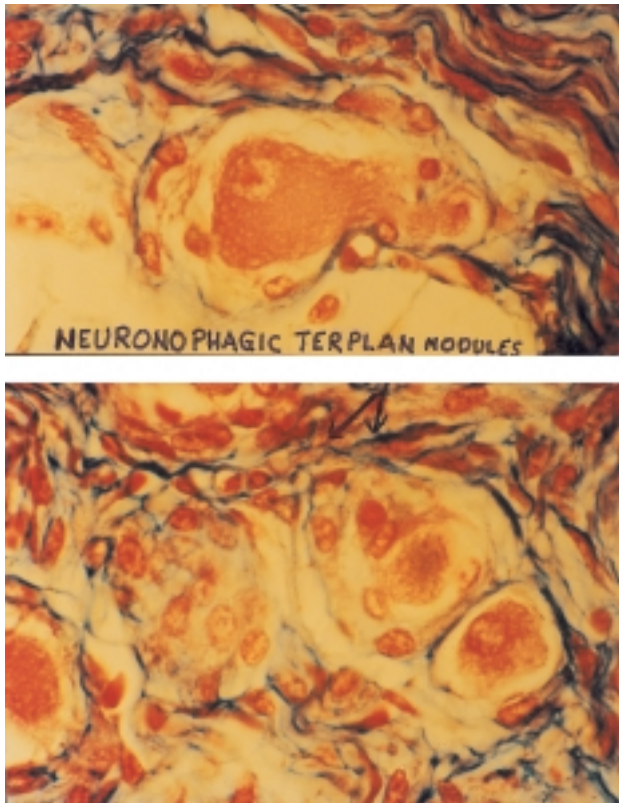

Figure 6. *Neuronophagia in transplanted hearts, clinically non-significant if limited (Azan 1000×).*

tion of the intrinsic nerves not only over a 2-5 cm wide area but up to the nerve terminals, and suggested to be crucial to the neuropathology of infarction.

Moreover, Zipes and Jalife's MIBG arguments in favor of an adrenergic imbalance in case of a myocardial infarction were shadowed by an adrenergic "denervation supersensitivity" masking the neural abnormality throughout the heart[5,11,12,16-20].

This, together with other personal interpretations were patently disproved by the book's neuroanatomist co-author (the world-famous W.C. Randall[8,14]), who still could not timely prevent the pitfall of two groups of academic cardiologists, who heedlessly chose MIBG to uphold[21,22] their neuropathological conclusions on right ventricular disease, methodologically impossible to ascertain by the MIBG procedure.

Anew, Randall and Ardell[14] with a much more sensitive set of techniques than MIBG-cum Phenol-brushing[4] went deeper into the complexity of the cardioneuroanatomy and pathophysiology, extending their research to small intensely fluorescent cells and interneurons[9,14].

An approximate quantitative evaluation of the neurophysiological derangements in case of myocardial infarction has been put forward[10,23]: the very rapid cardiac shift from aerobic to anaerobic oxygenation implies and the oxygen deprival drops from 38 molecules of adenosine triphosphate (produced by complete glucose oxidation) that cannot at all counterbalance the only 3 molecules converted from one glucosyl unit; so, no more than 7% of high energy phosphate would be available for the working myocardium; hence, in the infarcted area the heartbeat ceases. In the meantime acidosis and free radicals increase, while catabolite accumulation supervenes, enhancing arrhythmogenesis.

Arrhythmogenesis in infarction. Multifaceted pathological factors interplay in the anatomo-electrical derangement underlying arrhythmogenesis after myocardial infarction[11,17,18]; among these, the potassium efflux from myocytes and the release of endogenous epinephrine have been stressed[7,11].

Transplanted hearts. The overall question in transplanted hearts concerns their eventual reinnervation. This still cannot compare with the healing of an infarct[24].

The first prerequisite for spontaneous reinnervation consists of Schwann cell hyperplasia in the fashion of roughly aligned "Schwann tubules" (Fig. 5), predisposing the way from the proximal stump for new reformed nerve fibers to join the distal stump and reestablish the functional continuity. Surgical transplant cannot grant these conditions, but a tentative bilayered Schwannian growth was found close to the surgical disruption, thereby suggesting (but by no means proving) a theoretical possibility of reinnervation. The histological impression again arose that such a Schwannian growth was more pronounced (Fig. 5) in the atrial than in the ventricular stump and so as to prevail in the untransplanted segment of the new heart.

Discussion

An important semantic hallmark is the transplant's total loss of vagal afferent input, mainly in the left ventricle deputed to cardiopulmonary reflexogenesis; contrary to current beliefs[20,24], however, such a complete deafferentation cannot be compared at all with the incomplete one observed following a myocardial infarction. One should bear in mind that this diagnosis does not apply to the sympathetic input, because catecholamine neurotransmitters from extracardiac adrenergic sources, are still present in the circulation.

As far as the arrhythmogenic consequences of heart transplantation are concerned, conduction system abnormalities[25,26] predominate. Rowan and Billingham[27] maintained that regardless of function, parasympathetic neurons are never lost in long-term transplant survivors. Anyway, in heart transplants a cholinergic reinnervation is generally ruled out, whereas a regeneration of adrenergic terminals has recently been plausibly described[20]. However, details regarding the approximate location (recipient and donor's sinoatrial node plexuses) leave the neuromorphologist with ample margins of doubt. Overall, the major arrhythmogenic risks are conduction disorders[26].

Finally, with regard to the intracardiac ganglia, in transplanted hearts a variable number of Terplan nodules resulting from neuronal loss occurring prior to surgery may be seen[26], but they are clinically inconsequential.

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