

THE POTENTIAL LONG-TERM CARDIAC IMPLICATIONS OF
ANTECEDENT CRANIOCEREBRAL INJURY AND THE ROLE OF
CATECHOLAMINES IN THE PRODUCTION OF CARDIAC
HYPERTROPHY AND CARDIOMYOCYTE NECROSIS

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burg, in fulfilment of the requirements for the
degree of Master of Science in Medicineⁿ

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ABSTRACT

While donor hearts for cardiac transplantation have traditionally been harvested from head injured brain dead victims on the premise that these donors manifest neither macroscopic nor microscopic evidence of cardiac damage it has been shown both experimentally and clinically that brain death is associated with severe haemodynamic deterioration. However, it has also been shown that acute severe haemodynamic derangement can be fully reversed and that this, therefore, does not constitute an impediment or contraindication to transplantation of a donor heart. In contrast to the acute cardiac impairment which occurs in the brain death period immediately preceding cardiac transplantation, however, few studies have attempted to address the issue of the long-term cardiac implications of head injury, implications which may carry profound connotations both for the integrity of the transplanted heart and the recipient's future clinical cardiovascular status.

In an attempt to examine this issue, a prospective study was undertaken of all cases of cranio-cerebral injury coming to medicolegal autopsy over a six-month period in order to determine the nature of any histopathological changes which might arise following head injury. More importantly, the issue of whether any progression and evolution of these latter changes was explored, changes which might, with chronicity, carry potential implications for the patient's future cardiovascular status.

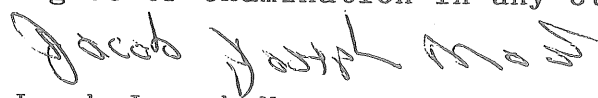
In this study the role played by catecholamines in the induction of cardiac hypertrophy and cardiomyocyte necrosis was explored together with the signaling mechanisms involved. In addition, in view of the effect of head injuries and intracranial lesions on the myocardium, the electrocardiographic abnormalities occurring in association with these lesions and mimicking

myocardial infarction and myocardial ischaemic changes was reviewed hopefully alerting clinicians to the presence of unsuspected intracranial pathology.

Statistical analysis indicated a statistically significant relationship between antecedent cranio-cerebral injury and subsequent myocardial fibrosis at a level of significance appropriate to exploratory research. Hence, it is suggested that all long-term survivors of head injury and, in particular, young survivors undergo routine follow-up, surveillance and monitoring of their clinical cardiovascular status in order to detect this potential complication which carries with it the risk of impairment of myocardial contractile function and, therefore, possible myocardial insufficiency. With regard to cardiac transplant recipients in whom donor hearts have been harvested from head injured brain dead victims, it is similarly suggested that they be monitored for the possible advent of cardiac decompensation on the basis of this latter complication in addition to standard monitoring for signs of transplant rejection.

DECLARATION

I declare that this dissertation is my own, unaided work. It is being submitted for the degree of Master of Science in Medicine in the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination in any other University.


Jacob Joseph Moar

10th day of March, 2008

DEDICATION

To my wife Helga
and
to my supervisor
Dr. Eric Quinton Klug, cardiologist

PREFACE

While it has long been recognised that donor hearts may develop rejection and coronary arteriosclerosis, as well as hypertension in the recipient, an issue which appears to have been inadequately addressed are the long-term implications of the cardiac histopathological changes developing after head injury. These changes may not only compromise hearts for transplantation purposes but may, if the heart is nevertheless transplanted into a recipient, carry with histopathological progression the potential for compromising the integrity of the transplanted heart and therefore the recipient's cardiovascular status.

In an attempt, therefore, to determine the nature of the histopathological changes developing after head injury which may, with progression, evolution and chronicity carry pathological implications with regard to the long-term integrity of a transplanted heart a prospective study of all cases of fatal craniocerebral injury coming to medicolegal autopsy at the State Mortuary, Johannesburg over a six-month period was undertaken.

This thesis is the outcome of that study. It is thus hoped that the findings emerging, as well as the attention drawn to the electrocardiographic changes which accompany intracranial pathology and which may mimic myocardial ischaemic changes and myocardial infarction may alert clinicians, in particular cardiologists and neurosurgeons, to both the immediate and long-term cardiac implications of antecedent craniocerebral injury. In so doing, it is further hoped that the survival and quality of life of the less severely head injured patient, in particular the young patient, as well as that of the cardiac transplant recipient, be enhanced.

Extracts from the methodology, test results

and discussion in chapters 7, 8 and 9 of the thesis were presented at the 15th Triennial Meeting of the International Association of Forensic Sciences held at UCLA, California, over 22-28 August, 1999, and were published in abstract form in the Proceedings of the IAFS, 1999: 196 and in Cardiovascular Pathobiology 1998; 2(3-4): 207-218 under the title 'Potential long-term cardiac implications of antecedent craniocerebral injury: a prospective study of 42 cases'.

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3. Dr. Peter King, Principal Specialist in the Department of Anatomical Pathology of the School of Pathology at the University of the Witwatersrand who reviewed the histopathology slides and assisted in selecting those deemed most appropriate for illustration and lesion demonstration;
4. Dr. S.G. Reinach (since deceased), biostatistician of the Institute of Biostatistics of the Medical Research Council and holder of a Ph.D. in biostatistics who performed the statistical analysis;
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unstinting assistance in the re-scanning of the Tables
and Figures.

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LIST OF ABBREVIATIONS

ATP	=	adenosine triphosphate
Ca ⁺⁺	=	calcium ion
CPK	=	creatine phosphokinase
cTnI	=	cardiac troponin I
DHD	=	donor heart dysfunction
DNA	=	deoxyribonucleic acid
ECG	=	electrocardiogram
GTP	=	guanosine triphosphate
HPF	=	high-power field
ICP	=	intracranial pressure
MRC	=	Medical Research Council
mRNA	=	messenger ribonucleic acid
μ	=	micron
Na ⁺	=	sodium ion
nm	=	nanometer
NMDGF	=	non-myocyte derived growth factor
NY	=	neuropeptide Y
PGF	=	prostaglandin F
PTAH	=	phosphotungstic-acid haematoxylin
SACs	=	stretch activated channels
SR	=	sarcoplasmic reticulum
TGF	=	transforming growth factor

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CHAPTER 1

THE ORIGINS AND BASIS OF CARDIAC TRANSPLANTATION AND THE POTENTIAL IMPLICATIONS OF USING DONOR HEARTS FROM HEAD-INJURED VICTIMS

While the first human heart transplant was carried out by Christiaan Barnard at Groote Schuur Hospital, Cape Town in December 1967,¹ the current most commonly used technique for cardiac transplantation originated with the work of Lower and Shumway in 1959.^{2,3} Historically, however, the father of human organ transplantation was Alexis Carrel who was awarded the Nobel Prize in 1912 for his pioneering work in vascular surgery.^{4,5} In a series of imaginative experiments, Carrel demonstrated that a heart could be transplanted and resume functioning in the new host, albeit in a non-orthotopic position. With the advent of techniques for successful cardiac surgery in the 1950's, attention could be directed to transplantation of the heart in the chest in the orthotopic, or normal, position.

Since then, advances in immunosuppressive therapy have permitted widespread application of cardiac transplantation with some 257 centres worldwide having performed this procedure in 1994 alone. Heart transplantation has since been extended to a wide variety of recipients, including neonates with hypoplastic left heart syndrome, patients with primary lung disease and elderly patients aged 60 to 70. However, while cardiac transplantation is thus established as treatment for end-stage cardiac failure, selection of donor hearts is nevertheless subjected to a number of exclusion criteria.

It has long been recognized that donor hearts may develop rejection⁶ and coronary arterio-

sclerosis, as well as hypertension in the recipient.⁷⁻⁹ However, an issue of paramount importance in view of the fact that donor hearts are harvested from irreperably brain damaged head injured victims and which appears to have been inadequately addressed are the long-term implications of the cardiac histopathological changes developing following head injury. These changes may not only compromise hearts for transplantation purposes but may, if the heart is nevertheless transplanted into a recipient, carry with lesion progression the potential for compromising the integrity of the transplanted heart and hence the recipient's cardiovascular status.

In an attempt, therefore, to determine the nature of the histopathological changes which may develop following fatal head injury and which may, with evolution and progression, carry pathological implications with regard to the long-term integrity of a transplanted heart, a study of all cases of fatal craniocerebral injury coming to medicolegal autopsy at the State Mortuary, Johannesburg over a six-month period was conducted on a prospective basis. All hearts taken at medicolegal autopsy were examined in accordance with a technique of cardiac mapping specifically devised to determine the presence of global cardiac pathology, an essential prerequisite for detecting what may be only patchy and focally distributed areas of pathological change.

While all histological slides from the 20 areas of myocardium sampled were subjected to histopathological examination by the author of the study, in order to provide standardisation and eliminate observer bias slides were periodically reviewed by a histopathologist holding Principal Specialist rank in the Department of Anatomical Pathology in the School of Pathology at the University of the Witwatersrand and whose particular expertise is cardiac pathology.

This latter pathologist similarly assisted in selecting those slides deemed most suitable and appropriate for photomicrographic illustration and lesion demonstration, acting in his capacity as previous supervisor when the thesis had originally been submitted for Ph.D.

A background and literature review of the early experimental findings in catecholamine-induced histopathological lesions in the myocardium reported in the English, German, and French literature since 1905 was conducted together with a brief description of cardiac organization and the conducting system of the heart in view both of the relationship between electrocardiographic abnormalities and intracranial lesions and their central nervous and cardiac morphological substrates resulting in ECG abnormalities which mimic myocardial infarction. The issue of intracranial lesions giving rise to ECG abnormalities of this nature was deemed to be of sufficient importance as to merit inclusion in a separate chapter.

The physiological basis and the pathophysiological correlation between the development and evolution of the histopathological lesions observed in the study and the underlying pathogenetic mechanisms in lesion progression were explored. In particular, the pathogenetic factors involved in the production of cardiac hypertrophy and cardiomyocyte necrosis following antecedent craniocerebral injury and its catecholamine storm were sequentially developed. These multiple pathogenetic mechanisms included, inter alia, the role of calcium ions in myocardial contractility and hypertrophic protein synthesis, autonomic control of the heart and the role of angiotensin II in signal transduction in the myocardium, the messenger systems involved, and the role of the contractile apparatus in end-stage myocardial dysfunction.

Two questions were essentially formulated in this exploratory study and subsequently subjected to statistical reduction and evaluation: i) is there a

myocardial change related to a scored craniocerebral injury that may be a predictor for survival outcomes in transplanted cardiac patients?, and; ii) is there a predictable significant change that occurs in the myocardium following a certain craniocerebral injury score? In this regard, statistical analysis was performed by a biostatistician from the Institute of Biostatistics of the Medical Research Council (MRC) and holder of a Ph.D. degree in biostatistics.

Conclusion

The pathological and statistical findings emerging from the study, as well as the attention drawn to the electrocardiographic changes which accompany intracranial pathology and which may mimic myocardial ischaemic changes and myocardial infarction may, it is hoped, alert clinicians in particular cardiologists and neurosurgeons, to both the immediate and long-term cardiac implications of antecedent craniocerebral injury. It is suggested that all long-term survivors of head injury and, in particular, young survivors undergo routine follow-up, surveillance and monitoring of their clinical cardiovascular status to detect any impairment of myocardial contractile function. With regard to transplant recipients it is similarly suggested that they be monitored for the possible advent of this complication in addition to standard monitoring for signs of transplant rejection. In so doing, it is thus hoped that the survival and quality of life of the less severely head injured patient, as well as that of the cardiac transplant recipient, be enhanced.

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CHAPTER 2

CARDIAC ORGANIZATION, THE CARDIAC CONDUCTION SYSTEM AND THE EARLY EXPERIMENTAL FINDINGS IN CATECHOLAMINE- INDUCED HISTOPATHOLOGICAL LESIONS IN THE MYOCARDIUM: BACKGROUND AND LITERATURE REVIEW

Cardiac Organization

The human heart is a pair of valved muscular pumps combined in a single organ. However, while the fibromuscular framework and conduction tissues of these pumps are structurally interwoven in a single complex functionally interdependent unit, each pump (the so-called 'right' and 'left' hearts) is physiologically separate. However, even this statement is an oversimplification as the two pumps have completely contrasting haemodynamic roles, and their outflow channels exhibit a mutual spiral of almost 180° as they discharge towards their primary destinations.

The higher peripheral resistance of the systemic circulation, together with the metabolic demands of the tissues supplied by this circulation, in particular the cerebral tissues, explains the more massive structural organization of the 'left heart' as opposed to the 'right heart'. This includes the geometry of the cavities, flow vectors, and the thickness of the left ventricular myocardium. Hence, the stresses imposed on the left ventricular wall are very much greater than those imposed on either the right ventricle or the atria and may explain the greater preponderance of lesions observed in the left ventricle in the study as opposed to the right ventricle or atria.

The Cardiac Conduction System

Vertebrate cardiac conduction originates in specialized

myocardial cells the nodes and networks of which constitute the cardiac conduction system (Figs. 2.1 and 2.2).¹ The system comprises the sinus node, the atrioventricular node, the atrioventricular bundle with its left and right bundle branches, and the subendocardial plexus of ventricular conduction cells (Purkinje fibres). In it the main pacemaker rhythm of the heart is generated being transmitted from atria to ventricles and thence within ventricular musculature.

Sinus Node

This, the cardiac 'pacemaker', initiates each cardiac cycle. It is located at the junction between parts of the right atrium derived from the embryonic venous sinus and the atrium proper extending between 1 and 2 cm on the right from the crest of the right auricle and runs postero-inferiorly into the upper part of the terminal groove.

Atrioventricular Node

This structure, located within the atrial component of the muscular atrioventricular septum, lies at the root of an extensive tree of conduction tissue reaching the apex of the ventricles, the papillary muscles and other ventricular regions (Fig. 2.1).

Atrioventricular Bundle

This structure is the direct continuation of the atrioventricular node. It branches on the crest of the muscular interventricular septum, dividing almost immediately into the left and right bundle branches that lie subendocardially on either side of the septum. The right bundle branch divides into fascicles at the origin of the anterior papillary muscle which then surround this

The conducting tissue of the heart seen from the right aspect

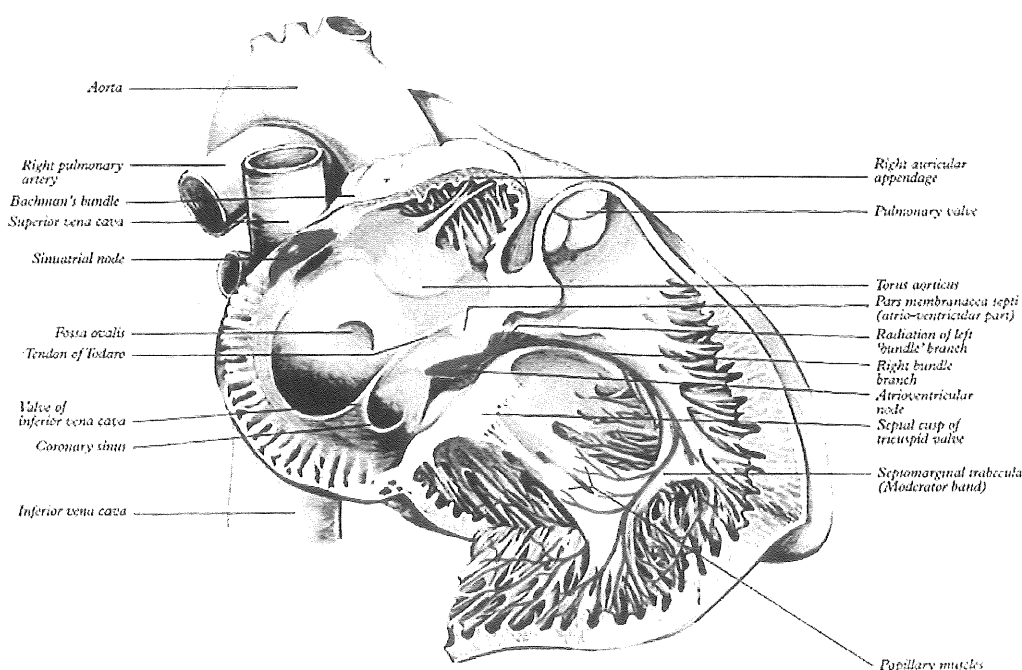


Fig. 2.1. A diagram of the conducting tissue of the heart as seen from the right aspect. Note the conducting tissue accompanying fine trabeculae carneae and false chordae. Note that in reality the radiation of the left bundle branch is directly related to the leaflets of the aortic valve. (Anderson RH. Co-ordination of cardiac activity: Conduction system. In: Gabella, G, ed. *Gray's Anatomy: The Anatomical Basis of Medicine and Surgery*. 38th ed. New York: Churchill Livingstone, 1995: 1495-1500).¹

The conducting tissue of the heart seen from the left aspect

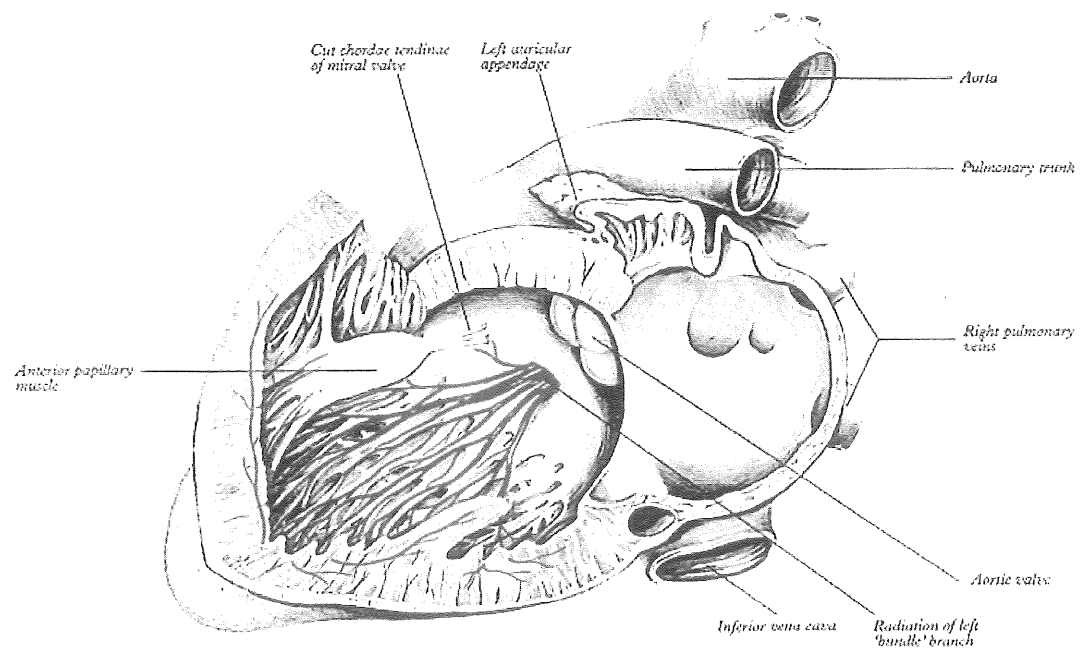


Fig.2.2. A diagram of the conducting tissue of the heart as seen from the left aspect. (Anderson RH. Co-ordination of cardiac activity: Conduction system. In: Gabella, G, ed. Gray's Anatomy: The Anatomical Basis of Medicine and Surgery. 38th ed. New York: Churchill Livingstone, 1995: 1495-1500).¹

muscle and then recurve subendocardially to the rest of the ventricular wall (Fig. 2.1). The left bundle branch, on the other hand, runs along the crest of the muscular interventricular septum separating into anterior, posterior and septal divisions which course back subendocardially to reach all parts of the left ventricle (Fig. 2.2).

Early experimental and clinical findings in catecholamine-induced histopathological lesions in the myocardium

While the injurious effects of catecholamines and their analogues on the myocardium have been known since the beginning of the twentieth century, it is nevertheless valid to examine these early experimental and clinical findings not only in view of the development, evolution and progression of the lesions of focal myocytolysis to stromal condensation and fibrosis noted in the study but also to provide the relevant background and literature review to the study.

As early as 1905 Ziegler, writing in the German literature,² reported the occurrence of intramyocardial haemorrhage, oedema, patchy areas of myofibrillar damage, round cell infiltration and, in particular, proliferation of fibrous tissue in the hearts of rabbits subjected to repeated injections of adrenaline. Ziegler found these lesions as early as six days after the onset of injections and noted areas of increased connective tissue and 'anaemic infarcts' after ten days of adrenaline administration. Pearce in 1906³ found similar lesions with myofibrillar oedema, loss of striations, and increase in fibrous tissue in the heart after seven to 15 injections of adrenaline. He considered the lesion to be related to constriction of the coronary arteries caused directly by adrenaline, of interest in view of the finding of contraction band

necrosis similarly emerging in the study. Such constriction was felt to be sufficient to interfere with augmentation of the blood supply that was needed by the more vigorously contracting myocardium. Pearce further noted that the lesions were similar to those found after ligation of a coronary artery.

During studies of induced cardiac hypertrophy Josue, writing in the French literature in 1907,⁴ noted myocardial degeneration and foci of sclerosis in rabbits who had received adrenaline intravenously in conjunction with subcutaneous injections of sterilized typhoid bacteria. Two years later, in 1909, Fleisher and Loeb produced similar lesions with either spartein or caffeine in combination with adrenaline.^{5,6} They found that production of the lesions was dependent on the adrenaline, but in the absence of the additional agents higher doses of adrenaline were required. Myocardial necrosis and fibrosis of the same type was found by Christian et al two years thereafter in 1911 while investigating the effects of adrenaline injections on the production of cardiac and renal injury.⁷

Further experimental investigations by other workers lead them to believe that ischaemia played a role in production of the catecholamine lesion.⁸⁻¹² Thus, Handforth, by injection of India Ink into the coronary arteries of hamsters sacrificed shortly after injection of isoproterenol, demonstrated that blood flow to the inner areas of the left ventricular wall was markedly reduced, even before development of myocardial oedema or necrosis. Handforth suggested that either localised vasoconstriction or shunting of blood to effectively bypass the subendocardial areas had taken place.

While from their electron microscopic observations Ferrans et al^{13,14} similarly felt that ischaemia was the apparent aetiology for the myocardial lesion caused by isoproterenol, these authors held in addition the view that the fibrosis found after isoproterenol

might be related to a direct stimulating effect of the drug on interstitial cell multiplication, an intriguing view in light of lesion progression from necrosis through stromal condensation to fibrosis noted in the study.

Pathophysiological mediation of the injurious effects of catecholamines on the myocardium

How might catecholamines mediate their injurious effects on the myocardium? While there are two concepts with regard to catecholamine cardiotoxicity i.e., i) the level of catecholamines, and; ii) the duration of level of catecholamines exerted, many authors have favoured ischaemia as the aetiological mechanism in the production of cardiac lesions. Through their marked inotropic stimulation, catecholamines induce ischaemia by producing disproportion between oxygen needs and oxygen availability. Cardiac automaticity is increased as manifested by an acceleration of the sinus rate and by the development of atrial and ventricular arrhythmias. Oxygen and substrate consumption by the heart is increased as is the amount of lipid available in the blood for use as fuel. In the cardiovascular system, the catecholamines lead to constriction and/or dilatation depending on the type of catecholamine and the specific receptor present. Thus, the pathogenesis of catecholamine cardiotoxicity is attributed to the relative hypoxia that occurs following direct stimulation of the myocardium, the coronary microcirculatory effect, membrane permeability alterations, catecholamine oxidation products (adrenochromes), and other contributory pathogenetic factors.¹⁵ Hence, although catecholamines have been used clinically for treatment of pump failure, excessive administration or release exceeding physiological doses may deplete the energy reserves of the cardiomyocytes leading to complex biochemical and subsequent structural changes progressing to necrosis.

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CHAPTER 3

THE ULTRASTRUCTURE OF CONTRACTILE CELLS AND THE ROLE OF CALCIUM IONS IN MYOCARDIAL CONTRACTILITY AND HYPERTROPHIC PROTEIN SYNTHESIS

The ultrastructure of the contractile cells

The contractile proteins of the heart lie within the muscle cells (cardiomyocytes) which constitute about 75 per cent of the total volume of the myocardium, although these comprise only about one-third in number of all the cells.¹ The individual cardiomyocytes account for more than half of the weight of the heart and are roughly cylindrical in shape. Those in the atria are quite small, less than 10 μ in diameter and about 20 μ in length. Relative to atrial cells, human ventricular myocytes are large, measuring about 17 to 25 μ in diameter and 60 to 140 μ in length.

When examined under the light microscope, the atrial and ventricular muscle cells have cross-striations and are branched. Each cell is surrounded by a complex cell membrane, the sarcolemma, and is filled with rod-like bundles of myofibrils. These latter comprise the contractile elements.

The sarcolemma of the myocyte invaginates to form an extensive tubular network (T-tubules) and which extends the extracellular space into the interior of the cell. As in skeletal muscle, this extension of the extracellular space in cardiac muscle serves to conduct the action potential into the depths of the cell.

Interspersed between the myofibrils and immediately beneath the sarcolemma are many mitochondria, the main function of which is to generate the energy in the form of adenosine triphosphate (ATP)

needed to maintain the heart's contractile function and the associated ion gradients. Indeed, about half of each ventricular myocyte is occupied by mitochondria illustrating the high energy requirements of this cell.

Of the other cellular organelles, the sarcoplasmic reticulum (SR) is the most important. This is a membrane-bound tubular plexus whose main role is the storage, release and re-accumulation of calcium ions. It comes into close contact with the T-tubules, leaving a 15nm gap spanned by structures termed either junctional processes or subsarcolemmal cisternae. These processes appear to be very similar biochemically, structurally and functionally to the ryanodine receptor-calcium release channels found in skeletal muscle at the junctional surface of the terminal cisternae. In cardiac muscle they initiate the contractile cycle by means of calcium release.²

The cytoplasm is the intracellular fluid and its proteins contained within the sarcolemma but excluding the contents of organelles such as mitochondria and the SR. The fluid component of the cytoplasm, minus the proteins, is called the cytosol. It is in the cytosol that the concentrations of calcium ions rise and fall to cause cardiac contraction and relaxation.

The role of calcium ions in myocardial contractility

The major function of cardiomyocytes lies in the contraction-relaxation cycle, the two chief contractile proteins involved in the contractile process being actin and myosin. Arranged at regular intervals along both sides of the actin filament are the regulatory proteins troponin and tropomyosin. Troponin consists of a complex of three sub-units of which troponin-C, as its name indicates, is calcium-binding and which responds to the calcium ions that are released from

the SR to start the contractile cycle. When sufficient calcium is present myosin interacts with actin initiating a process known as crossbridge cycling. Calcium ions play an indispensable role in this process by providing a crucial switch-on signal to the crossbridge cycle.

Crossbridges exist in either a strong or weak binding state.³ As long as enough calcium ions are present, the strong binding state potentially dominates. When, however, cytosolic calcium levels fall at the start of diastole, a master switch is turned off as the calcium ions leave troponin-C; tropomyosin then assumes an inhibitory configuration and the weak binding state predominates.⁴

Calcium ions stimulate the contractile process at multiple control sites and their interaction with troponin-C is essential for crossbridge cycling.⁵⁻⁷ The mechanism of calcium action on myofilament proteins affecting both the extent and rate of force development and hence the contractility of the myocardium has been explained on the basis of geometrical shifts, analogous to that in ligand-gated ion channels,⁸ the extent of activation depending on the free calcium ion concentration.

In addition to the model explaining the rate of force development (and hence myocardial contractility) on the basis of geometrical shifts, two further models have been proposed to account for increased force development at greater calcium ion concentrations. Firstly, it has been held that calcium acts as an on-off switch and that therefore the increased force development in the presence of calcium ions must be due to recruitment of additional crossbridge sites.⁹ The alternative viewpoint is that the crossbridges react to calcium in a graded manner.⁵⁻⁷ These biomolecular models governing the extent and force of myocardial contraction are intriguing in light of the predominant involvement of the left ventricle, the

most forcefully and actively contractile part of the heart, by the pathological lesions emerging in the study (atrial contraction only produces the last 20 to 30 per cent of ventricular filling the initial 70 per cent of venous blood entering the ventricles directly prior to atrial contraction).

The role of calcium ion in cellular injury and hypertrophic protein synthesis.-----

A complex interplay exists between the two major regulators of cardiac function- Ca^{++} and stretch.¹⁰ Stretch has been shown to alter action potential configuration, generate stretch-activated arrhythmias, and increase the rate of beating of the sino-atrial node. A variety of mechanisms including Ca^{++} entry through stretch-activated channels (SACs), and mobilisation of intracellular Ca^{++} stores have been proposed to account for the effect of stretch on rhythm.

Stretch may initiate a series of conformational changes in ion channels or receptors to allow increased entry of a specific ion, such as Na^+ , which can then be converted to a calcium signal by $\text{Na}^+/\text{Ca}^{++}$ exchange. The pathological significance of this pathway/mechanism is that accumulation of intracellular Na^+ activates the $\text{Na}^+/\text{Ca}^{++}$ exchanger with resultant Ca^{++} overload. Specifically, elevated free Ca^{++} concentrations cause cellular injury during hypoxia/ischaemia leading to irreversible myocardial damage during reoxygenation/reperfusion. $\text{Na}^+/\text{Ca}^{++}$ exchange thus plays a crucial role in cellular injury during hypoxia/ischaemia.

Ca^{++} also appears to be important in the pathway that leads to expression of precursors of hypertrophic protein synthesis. Of particular interest is the concept that an early event in the sequence leading from muscle stretch to hypertrophy is release of angiotensin II from the stretched muscle.¹¹ Angiotensin II opens Ca^{++} channels in rabbit myocardium¹²

and release of angiotensin II may play a major role in the regulation of cardiomyocyte growth and hence be of relevance to the development of the cardiomyocyte nuclear changes of hypertrophy noted in the study. Indeed, it is recognised that stretch-induced release of angiotensin II stimulates cardiac mRNA formation as, for example, in production of the cardiac renin gene.^{13,14}

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CHAPTER 4

CARDIAC RECEPTORS, AUTONOMIC CONTROL OF THE HEART AND THE ROLE OF ANGIOTENSIN II IN SIGNAL TRANSDUCTION IN THE MYOCARDIUM

Signal transduction is the sum total of processes whereby an extracellular hormonal or neural stimulus is converted to an intracellular physiological change. Adrenergic or cholinergic stimulation of the sarcolemmal receptors inaugurates the activity of a complex system of sarcolemmal and cytosolic messengers.¹ For example, occupancy of the beta-adrenergic receptor is coupled to activation of a sarcolemmal enzyme, adenylylate cyclase, by a G-protein complex.^{2,3} The G-protein complex involved, being stimulatory, is called G_s .³ Adenylylate cyclase produces a second messenger, cyclic AMP, which then acts through a third messenger, protein kinase A, to increase cytosolic calcium transients.⁴ A similar messenger system in the sinus node increases the heart rate. Cholinergic stimulation, on the other hand, exerts inhibitory influences, largely on the heart rate but also on atrial contraction, acting at least in part by decreasing the rate of formation of cyclic AMP.

Autonomic control of the heart

While initiation of the cardiac cycle in vertebrates originates unequivocally in specialized myocytes, neural influences play a major role not only in cardiac adaptation to the functional demands of the body but also in the response to injury. For example, the hypothalamus although comprising only 4 cm³ of neural tissue, or 0.3 per cent of the total brain, can, through its actions on the vasomotor centre produce

every known type of neurogenic effect on the cardiovascular system and, in particular, massive adrenergic discharge following head injury. Conversely, nor-adrenergic cell groups in the brainstem reticular formation project rostrally as a major catecholamine pathway which terminates in the amygdala and hypothalamus.⁵

Peripheral autonomic activity is integrated at higher levels in the brainstem and cerebrum, including various nuclei of the brainstem reticular formation, the thalamus, hypothalamus, limbic lobe and prefrontal neocortex, together with the ascending and descending pathways which interconnect these regions. It is recognized that central control of the cardiovascular system is exerted by a longitudinally arranged series of parallel pathways involving specific regions of the neuraxis extending from the cerebral cortex to the spinal cord.⁶ The autonomic nervous system, with both afferent and efferent components, thus acts on the nodal tissues and their prolongations, on coronary vessels, and on the working atrial and ventricular myocardium. Rich plexuses of nerves containing cholinesterase, adrenergic transmitters, and other peptides such as neuropeptide Y (NY) are found in the subendocardial regions of all chambers and in the valve leaflets.⁷ Complex endorgans have also been described in the subendocardial region of the left atrium,⁸ and studies in tissue culture have shown that ganglion cells cause constriction of the coronary arteries of relevance in development of contraction band necrosis. These intrinsic ganglia act as sites for integration not only of extrinsic nervous inputs forming complex circuits for the local neuronal control of the heart but also local reflexes such as coronary artery constriction which may develop after head injury.⁹

Alpha-Adrenergic Receptors and the messenger systems involved in controlling Ca^{++} flux and vascular constriction

In contrast to the G-protein complex associated with the beta-adrenergic receptor, other cardiac receptors such as the α_1 -adrenergic receptor, have an alternate dual messenger system involving inositol trisphosphate and diacylglycerol, with the latter activating protein kinase C.^{10,11} Such signals are of established importance in controlling calcium flux in vascular smooth muscle thereby regulating vascular tone with its attendant constriction.

Research findings have illustrated the complexity of the signal transduction pathways linked to α_{1A} -adrenoceptors.¹² All α_1 -adrenoceptor subtypes have been shown to couple to phospholipase C which results in the release of intracellular Ca^{++} . Furthermore, a given α_1 -subtype may be coupled to a variety of different signal transduction mechanisms in different systems. Moreover, there may be different effector mechanisms linked to α_1 -subtypes in a given cell or tissue, i.e. heart as opposed to prostate.

Beta-Adrenergic Receptors and the role of catecholamines and Ca^{++} in cardiac contractility

The triple combination of the beta-receptor, the G-protein complex, and adenylate cyclase is termed the beta-adrenergic system.¹³ Cardiac beta-adrenergic receptors are chiefly the β_1 -subtype, whereas most noncardiac beta-receptors are β_2 . However, β_2 -receptors also occur in the human heart, constituting about 20 per cent of the total beta-receptor population in the left ventricle and about 40 per cent in

the atria.¹⁴ These β_2 -receptors appear to have greater efficacy in their capacity to activate the G-protein-adenylate cyclase system than do the β_1 -receptors.¹⁵

The most potent physiological means of enhancing cardiac contractility is through the action of beta-adrenergic agents such as the naturally occurring catecholamines, epinephrine and norepinephrine. These act by keeping the L-type Ca^{++} channel open for longer increasing the amount of Ca^{++} entering during depolarization,¹⁶ and by enhancing the activity of the Ca^{++} -pumping ATPase by phosphorylating phospholamban, a protein acting as the major regulator of the Ca^{++} pump.¹⁷ This is brought about by cyclic AMP-dependent protein kinase.

The sequence of events describing the positive inotropic effects of catecholamines is as follows¹⁸:

Catecholamine stimulation $\longrightarrow$ beta-receptor $\longrightarrow$ molecular change $\longrightarrow$ binding of GTP to α_s -subunit of G-protein $\longrightarrow$ GTP- α_s -subunit stimulates adenylate cyclase $\longrightarrow$ formation of cyclic AMP from ATP $\longrightarrow$ activation of cyclic AMP-dependent protein kinase $\longrightarrow$ phosphorylation of a sarcolemmal protein p27 $\longrightarrow$ increased entry of Ca^{++} through increased opening of the voltage-dependent L-type calcium channels $\longrightarrow$ greater calcium-induced calcium release via ryanodine receptor of sarcoplasmic reticulum $\longrightarrow$ greater rise of intracellular free Ca^{++} concentration $\longrightarrow$ increased calcium-troponin-C interaction with deinhibition of tropomyosin effect on actin-myosin interaction $\longrightarrow$ increased rate and number of crossbridges interacting with increased myosin ATPase activity $\longrightarrow$ increased rate and peak force development (Figs. 4.1 and 4.2).

Signal systems involved in beta-adrenergic stimulation

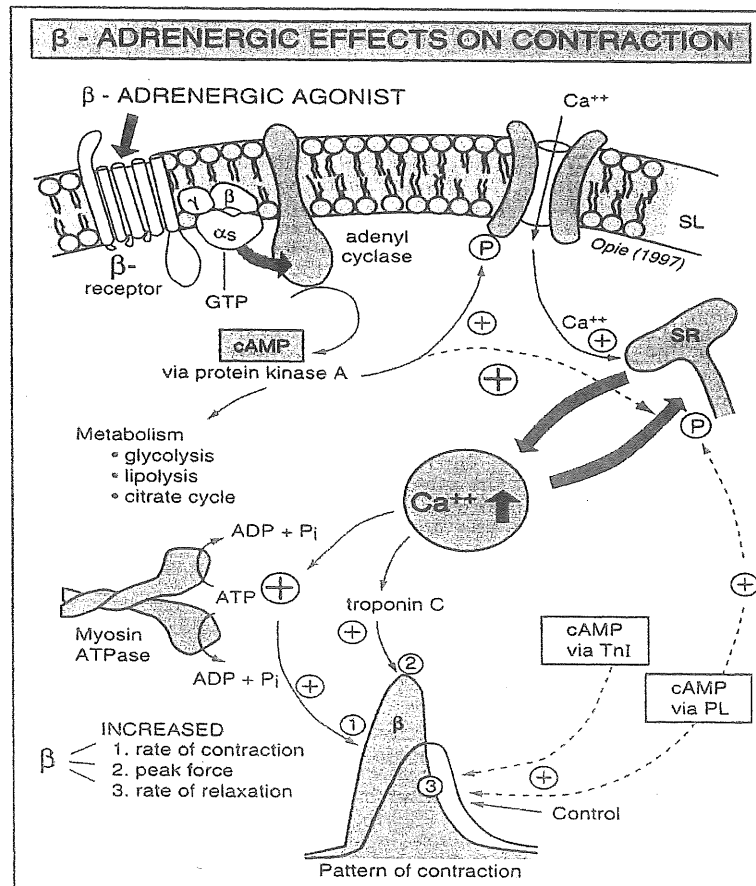


Fig. 4.1. Signal systems involved in positive inotropic and lusitropic (enhanced relaxation) effects of beta-adrenergic stimulation. When the beta-adrenergic agonist interacts with the beta-receptor, a series of G-protein-mediated changes lead to activation of adenylate cyclase. (Copyright @1997 L.H Opie).¹⁸

Ca^{++} ions in the contractile process

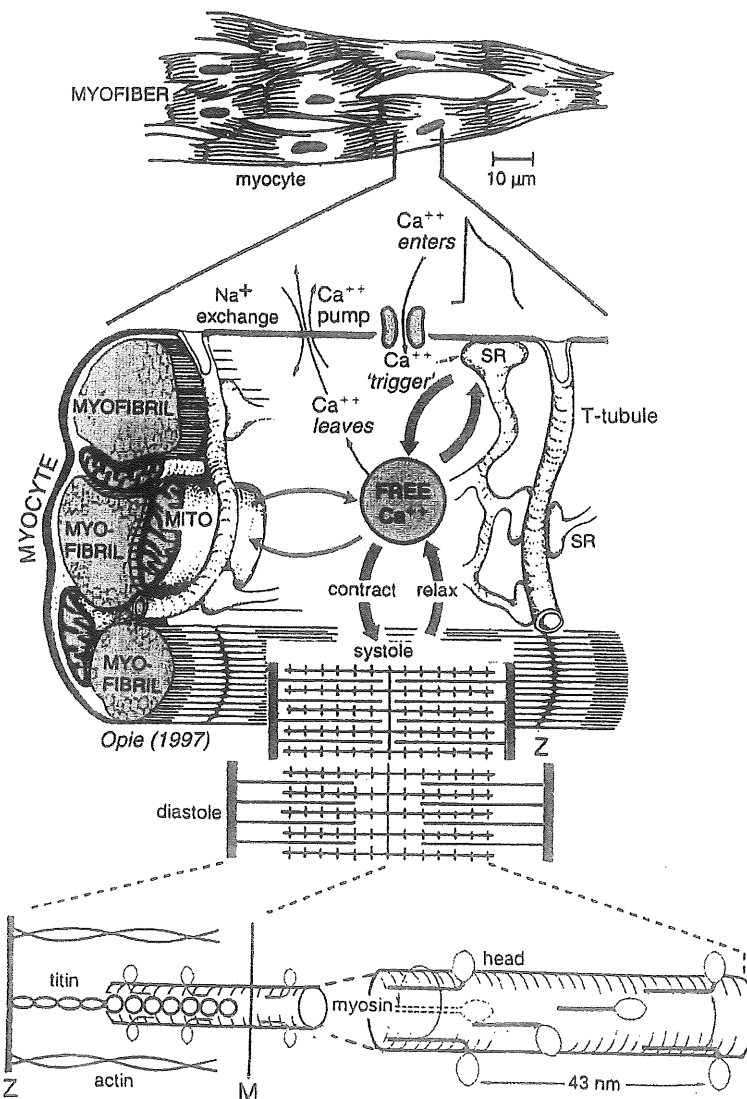


Fig 4.2. The crux of the contractile process lies in the changing concentrations of Ca^{++} ions in the myocardial cytosol. Ca^{++} ions are schematically shown as entering via the calcium channel, which opens in response to the wave of depolarization that travels along the sarcolemma. These Ca^{++} ions

trigger the release of more calcium from the sarcoplasmic reticulum (SR) and thereby initiate a contraction-relaxation cycle. Eventually the small amount of calcium that has entered the cell leaves, predominantly by a $\text{Na}^+/\text{Ca}^{++}$ exchanger with a lesser role for the sarcolemmal calcium pump. The varying actin-myosin overlap is shown for systole and diastole. The myosin heads, attached to the thick filaments, interact with the thin actin filaments. (Copyright © 1997 L.H. Opie.)¹⁸

Cholinergic Receptors, signal transduction and the 'braking' of beta-adrenergic stimulation

In the case of the parasympathetic system, signaling is again via an extracellular first messenger (acetylcholine), a receptor system (the muscarinic receptor), and a sarcolemmal signaling system (the G-protein system). The myocardial muscarinic receptor (M_2) is specifically associated with the activity of the vagal nerve endings. Receptor stimulation produces a negative chronotropic response.

The mechanism of the negative inotropic effect of vagal stimulation is multiple, and includes: 1: bradycardia; 2: inhibition of cyclic AMP formation; and 3: a direct negative inotropic effect mediated by cyclic GMP (Fig. 4.3).¹⁹ Ventricular tissue is much less responsive to muscarinic agonists than atrial tissue, although the receptor populations are of similar density. Thus, postreceptor differences must exist, probably in the degree of G-protein coupling. In general, the negative inotropic effect has best been observed in the presence of beta-stimulation, when vagal effects counteract those of prior beta-stimulation.²⁰

Interaction between parasympathetic and sympathetic systems at a cellular level

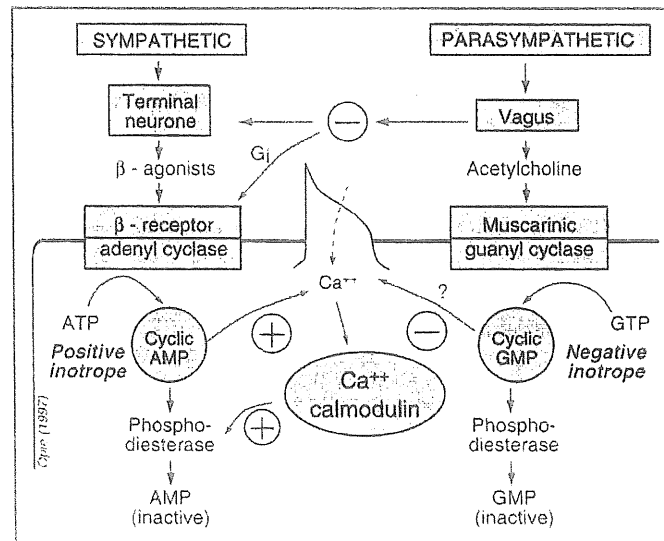


Fig. 4.3. Interaction between parasympathetic and sympathetic systems at a cellular level may involve two opposing cyclic nucleotides, cyclic AMP and cyclic GMP. Many effects of vagal stimulation could best be explained by the inhibitory effect on the formation of cyclic AMP, including formation of inhibitory G-protein, G_i , in response to M_2 -receptor stimulation. (Copyright @ 1997 L.H Opie)¹⁸

A further mechanism for parasympathetic-sympathetic interaction lies at the level of the sympathetic terminal neurons, where a presynaptic muscarinic M_2 -receptor inhibits the release of norepinephrine.

In addition to the above, both adrenergic and cholinergic stimuli exert complex and potentially important effects on ion channels that can be translated into opposing effects on cardiac function. The presence of such multiple mechanisms for the inhibitory effects of vagal stimulation on the heart rate and inotropic state suggest that 'braking' of beta-adrenergic stimulation is physiologically desirable. Otherwise, the risk may be that intense beta-adrenergic stimulation would excessively increase the heart rate or inotropic state.

The role of angiotensin II in signal transduction and cardiomyocyte growth

Research using a rat heart model has indicated a role for angiotensin II in intracellular signaling,²¹ following on earlier research elucidating the role of angiotensin II signal transduction pathways in the cardiovascular system.²² In the case of cardiomyocytes, it is currently appreciated that receptors such as angiotensin II coupled to protein kinase C, may play a major role in the regulation of cardiomyocyte growth, of relevance to the findings of nuclear hypertrophy in the study.

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CHAPTER 5

END-STAGE MYOCARDIAL DYSFUNCTION REQUIRING CARDIAC TRANS- PLANTATION AND THE ROLE OF CALCIUM AND THE CONTRACTILE APPARATUS

Excitation-Contraction Coupling and the role of Ca^{++}

As previously indicated, Ca^{++} plays a pivotal role in the regulation of myocardial contraction and relaxation. Ca^{++} has been shown to augment contractility in patients with severe heart failure secondary to cardiomyopathy who have downregulation of beta-adrenergic receptors.¹

Myocardium obtained at the time of cardiac transplantation from patients with end-stage heart failure exhibits abnormal prolongation of the action potential and developed force, and impaired relaxation.² Observations using the Ca^{++} indicator aequorin in whole myocardium have shown that these alterations in electrical and contractile properties are associated with a prolonged elevation of the intracellular Ca^{++} transient during relaxation.³ Likewise, in myocytes obtained from patients with end-stage heart failure, the action potential is prolonged. The intracellular Ca^{++} transient, as assessed by the fluorescent indicator fura-2, demonstrates a blunted rise with depolarization reflecting a slower delivery of Ca^{++} to the contractile apparatus (causing slower activation) and a slowed rate of fall during repolarization (causing slowed relaxation) (Fig. 5.1).⁴ These two abnormalities can explain in part both systolic and diastolic dysfunction although it is in actual fact more complicated than that.

Abnormal action potential and intracellular calcium transient in failing cardiomyocytes

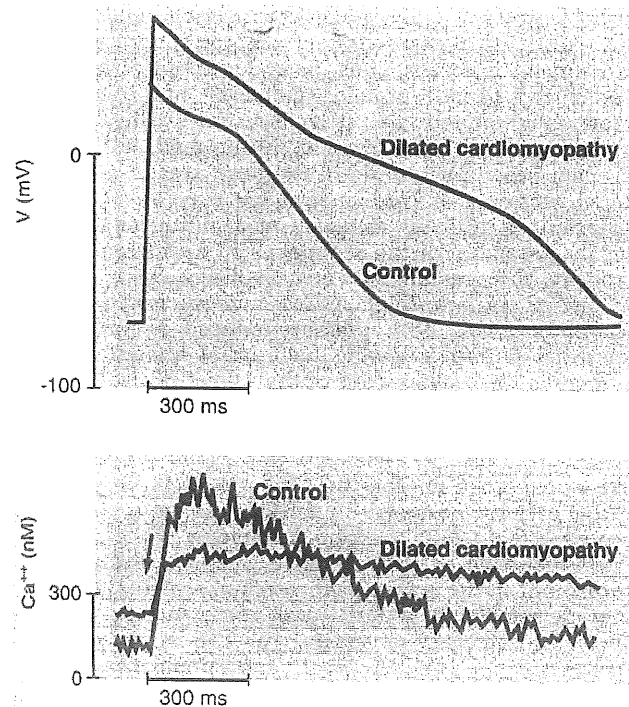


Fig. 5.1. Top panel: The action potential recorded in a myocyte isolated from the heart of a patient with dilated cardiomyopathy is markedly prolonged as compared to that in a myocyte from a normal heart (control). Such abnormalities could contribute to both the generation of arrhythmias and abnormal diastolic relaxation. Bottom panel: The intracellular calcium transient measured with the fluorescent calcium indicator fura-2 is also markedly abnormal in myocytes isolated from the myocardium of patients

with dilated cardiomyopathy. As compared to a normal myocyte (control), the myocyte from a patient with dilated cardiomyopathy shows an attenuated rise with depolarization (arrow) and a markedly delayed return to baseline. These abnormalities reflect the altered expression or function of key calcium handling proteins (i.e., Ca^{++} -ATPase) and likely contribute to the abnormal action potential. (Beuckelmann DJ, Nabauer M, Erdmann E. Intracellular calcium handling in isolated ventricular myocytes from patients with terminal heart failure. *Circulation* 1992; 85: 1046-1055. Copyright 1992 American Heart Association.)⁴

Additional evidence of abnormal myocardial Ca^{++} handling is provided by the observation that there is a reduction in the amount of tension-independent heat produced in myocardium from patients with heart failure.⁵ Tension-independent heat, which is believed to reflect the energy expended for Ca^{++} transport, can be used to estimate the amount of Ca^{++} cycled per heartbeat. Using this approach, it has been shown that Ca^{++} cycling is reduced by approximately 50 per cent in failing human myocardium.⁶

Sarcoplasmic Reticulum Ca^{++} reuptake reduction in end-stage cardiac failure

Ca^{++} reuptake by the SR is mediated primarily by the ATP-dependent enzyme Ca^{++} -ATPase (also called SERCA2) and which constitutes nearly 90 per cent of the protein component of the SR. The activity of SERCA2 is inhibited by the associated protein phospholamban.^{7,8} This inhibition is opposed by

cyclic AMP-mediated phosphorylation of phospholamban (i.e., by beta-adrenergic receptor stimulation) thereby resulting in increased Ca^{++} reuptake into the SR and the augmentation of diastolic relaxation. The reuptake of Ca^{++} by the SR is also important for normal systolic function, which requires that sufficient SR Ca^{++} be available for release during systole to mediate contraction.

Reports have indicated that the levels of SERCA2, phospholamban mRNA and SERCA2 protein are reduced in myocardium obtained from patients with end-stage heart failure.⁹⁻¹² In one study,¹⁰ the decrease in the level of SERCA2 mRNA was inversely related to the level of atrial natriuretic factor mRNA, suggesting that the reduced expression of this adult muscle-specific protein is coupled to the re-expression of a foetal gene program. These observations are consistent with several studies that have demonstrated a reduction in SR Ca^{++} reuptake in animal models of heart failure¹³⁻¹⁵ and some^{16,17} but not all¹⁸ studies in human heart failure patients. Mice deficient in phospholamban due to targeted gene ablation have shown a marked increase in basal myocardial contractility and a loss of both the contractile and relaxant effects of beta-adrenergic stimulation. It is possible, therefore, that a decrease in phospholamban activity is an adaptive response to the decrease in SERCA2 activity.

The Ca^{++} Release Channel in failing human myocardium

The Ca^{++} release channel located on the SR mediates Ca^{++} release from the SR into the sarcoplasm during systole. As indicated above, and at the risk of over-emphasis, Ca^{++} plays a pivotal role in animal models of heart failure and some but not all studies of human heart failure patients but carrying, however,

implications for left ventricular dysfunction and ventricular remodeling following injury and haemodynamic stress, of relevance to the findings in the study. Research of failing human myocardium has shown decreases in the mRNA level for the Ca^{++} release channel with a reduction in the mRNA level in patients with ischaemic heart disease but not in those with dilated cardiomyopathy.¹⁹ Another study, quoted above,¹⁰ showed mRNA reduction in patients with heart failure from both causes. The mRNA and protein levels of the voltage-dependent Ca^{++} channel have also shown to be decreased in failing human myocardium obtained from patients with both ischaemic heart disease and dilated cardiomyopathy.²⁰

The $\text{Na}^+/\text{Ca}^{++}$ Exchanger and its role in increased arrhythmogenicity in failing human myocardium

The $\text{Na}^+/\text{Ca}^{++}$ exchanger is the major route by which Ca^{++} is removed from the cardiomyocyte and can account for approximately 20 per cent of the Ca^{++} removed from the cytoplasm during diastole. In failing human myocardium however caused, the mRNA and protein levels of the Na/Ca^{++} exchanger have been found to be increased, correlating inversely with the decrease in SERCA2 mRNA levels described above.¹¹ This augmentation in $\text{Na}^+/\text{Ca}^{++}$ exchange activity might be a compensatory response to the reduction in Ca^{++} reuptake caused by a decrease in SERCA2. While this would facilitate diastolic Ca^{++} removal, it might do so at the expense of increased arrhythmogenicity, as this Ca^{++} efflux is associated with an influx of Na^+ that can prolong depolarization and cause afterdepolarizations. On the other hand, with regard to calsequestrin the major protein in the SR that binds Ca^{++} thereby serving a storage function, several studies quoted above in relation to SR Ca^{++} -

ATPase have found calsequestrin mRNA levels to be unchanged in failing human myocardium.^{10,18,20}

The Contractile Apparatus in left ventricular systolic failure and left ventricular hypertrophy

Patients with left ventricular systolic failure show a significant reduction in cell volume occupied by myofibrils.²¹ Electron microscopic observations show a reduction of ventricular myofibrillar protein,²² suggesting that this reduction in the contractile machinery may play a role in the development of or occur as a consequence of cardiac decompensation.²³ In left ventricular hypertrophy on the other hand, animal models have shown that the fraction of cell volume composed of myofibrils is initially increased.²³ Animal studies have further shown that when the adult heart hypertrophies, foetal and neonatal forms of contractile proteins (termed isoforms) and other proteins (such as atrial natriuretic peptide) reappear, signifying reexpression of the genes for these foetal and neonatal isoforms.^{24,25} In rodents, for example, the predominant myosin heavy chain (MHC) is the 'fast' V₁ isoform. With pressure-induced hypertrophy, there is reexpression of the 'slow' V₃ isoform.^{26,27} Thus, haemodynamic overload leads to enhanced overall protein synthesis,²⁸ in addition to altering the proteins qualitatively, i.e., it induces the synthesis of protein isoforms that were present during foetal and neonatal life when protein synthesis in the heart was also rapid.²⁹

The predominant MHC isoform in humans is the slower V₃ isoform, encoded by the beta-MHC gene. In failing human human myocardium beta-MHC, as indicated, is reduced,¹⁰ while it has further been reported that a reduction in the number of cells containing alpha-MHC also occurs.³⁰

It has further been observed that in rats with cardiac hypertrophy, changes in the myosin light chain and the troponin-tropomyosin complex occur,^{31,32} while in humans changes in myosin light chain isoforms have been observed in the atria and ventricles of patients subjected to increased mechanical stress.³³ These constitute part of the cardiac adaptive responses to the myocardial dysfunction of terminal cardiac failure or those following the catecholamine-induced haemodynamic stress of craniocerebral injury.

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CHAPTER 6

THE RELATIONSHIP BETWEEN ELECTROCARDIOGRAPHIC ABNORMALITIES AND INTRACRANIAL LESIONS AND THEIR CENTRAL NERVOUS AND CARDIAC MORPHOLOGICAL SUBSTRATES

Early studies of the relationship between intra-
cranial lesions and electrocardiographic abnor-
malities

Acute cerebral injury can provoke cardiovascular abnormalities while abnormalities of the heart may set the stage for acute cerebral injury. A relationship between certain acute cerebral events such as intracerebral haemorrhage or subarachnoid haemorrhage has been recognized for almost a century, with a relationship between head injury and cardiac abnormalities being proposed more than 70 years ago.¹ Almost 70 years ago, in 1938, Aschenbrenner and Bodechtel, writing in the German literature, reported that neurological lesions such as brain tumours could be associated with electrocardiographic abnormalities and, moreover, in young patients without heart disease.² Experimental work in animals before this had, however, already suggested that the heart might be adversely affected by central nervous system influences. It had been shown in 1937 that vagal stimulation in dogs could cause electrocardiographic changes, signs of cardiac decompensation, and myocardial infarction.³

Following the study by Aschenbrenner and Bodechtel, Byer, Ashman and Toth reported in 1947 that electrocardiographic abnormalities could be observed in patients following subarachnoid haemorrhage, and that these changes consisted of giant inverted T waves and prolonged Q-T intervals.⁴ A more detailed and systematic study, published in

1954 by Burch,⁵ and confirmed by other workers,⁶⁻⁹ concluded that the principal offending lesions were intracerebral and subarachnoid haemorrhage, and that the principal electrocardiographic abnormalities were prolongation of the Q-T interval, increased amplitude and duration of T waves and abnormal U waves (Figs. 6.1, 6.2 and 6.3).

Approximately 90 per cent of patients with acute cerebrovascular accidents, especially intracerebral or subarachnoid haemorrhage, acute craniocerebral injury, and peripheral or central nervous system manipulative procedures, exhibit electrocardiographic abnormalities that consist chiefly of disturbances of cardiac rhythm and repolarization.^{1,6-30} Abnormalities of rhythm include sinus bradycardia (defined as ≤ 50 beats/minute), sinus tachycardia (defined as ≥ 100 beats/minute), atrial arrhythmias (ectopic atrial beats, atrial fibrillation, atrial flutter, or other supraventricular tachycardias), junctional rhythms, and ventricular arrhythmias (ventricular tachycardia, ventricular ectopic beats, or ventricular fibrillation).

The finding of a prolonged Q-T interval in subarachnoid haemorrhage by Hersch, one of the authors referred to above,⁹ accords with previous observations by the same author in cases of head injuries,³⁰ in which the Q-T interval was prolonged in 15 per cent of patients. Of further interest is that as the level of consciousness deteriorated, the number of electrocardiographic abnormalities increased.

Spontaneous intracranial haemorrhage with T-wave inversion in a 40-year-old male

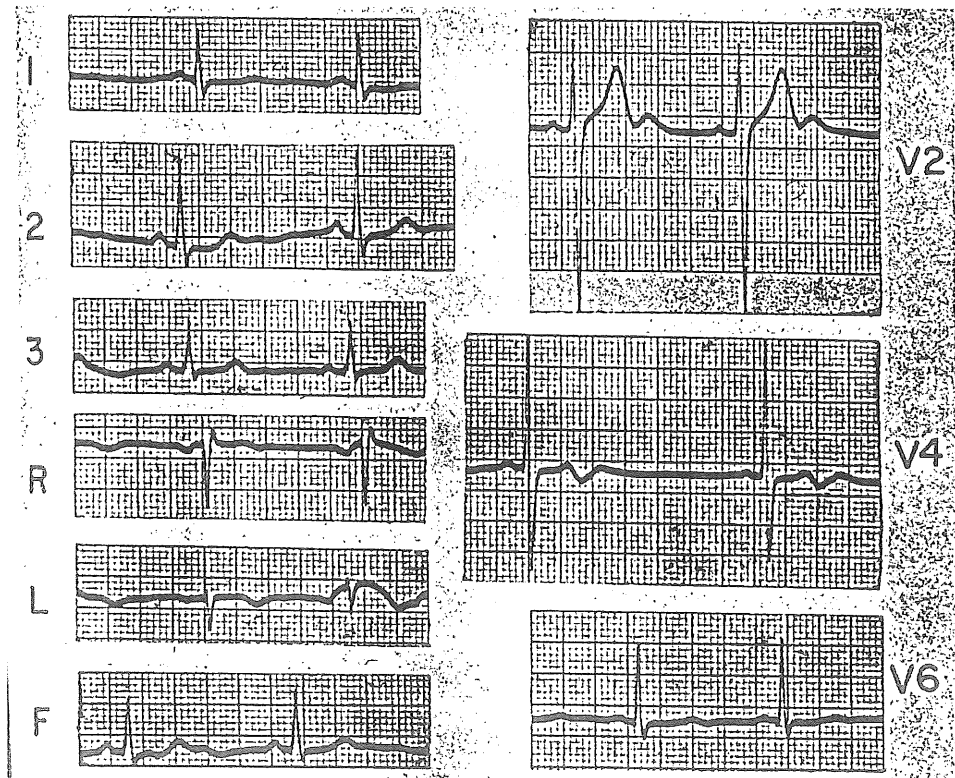


Fig. 6.1. Spontaneous intracranial haemorrhage in a 40-year-old male.

Electrocardiogram shows abnormally large U waves particularly in V₁ and V₄ along with T-wave changes suggestive of myocardial ischaemia. (Cropp GJ, Manning GW. Electrocardiographic changes simulating myocardial ischaemia and infarction associated with spontaneous intracranial haemorrhage. *Circulation* 1960; 22: 25-38.)⁸

Spontaneous intracranial haemorrhage with T-wave inversion in a 55-year-old female.

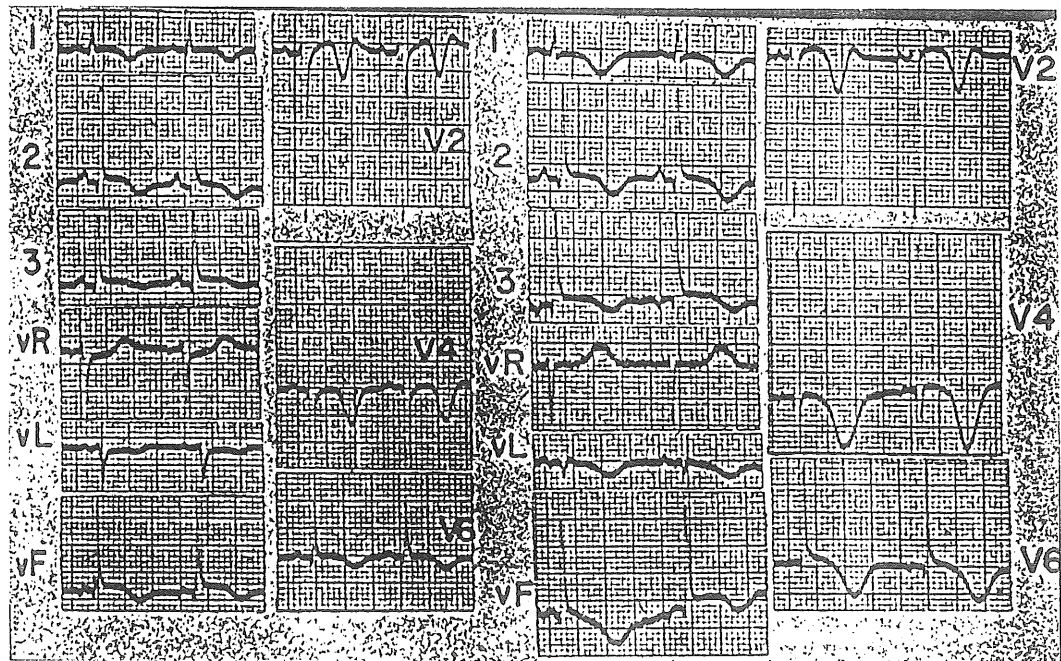


Fig. 6.2. Spontaneous intracranial haemorrhage in a 55-year-old female.

Electrocardiograms taken shortly after onset of haemorrhage show a pattern of recent myocardial infarction. Note the T-wave inversion. Postmortem findings disclosed normal heart and coronary arteries (Cropps GJ, Manning GW.

Electrocardiographic changes simulating myocardial ischaemia and infarction associated with spontaneous intracranial haemorrhage. *Circulation* 1960; 22: 25-38.)⁸

Spontaneous intracranial haemorrhage with electrocardiographic changes
suggestive of myocardial damage

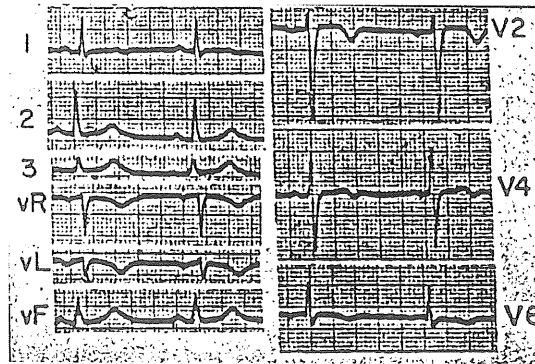


Fig. 6.3. Spontaneous intracranial haemorrhage in a 51-year-old male. Borderline electrocardiographic changes suggestive of myocardial damage. (Cropp GJ, Manning GW. Electrocardiographic changes simulating myocardial ischaemia and infarction associated with spontaneous intracranial haemorrhage. *Circulation* 1960; 22: 25-38.)⁸

Central nervous system loci involved in autonomic responses and the production of electrocardiographic abnormalities

Many experimental investigations have been carried out in an attempt to determine the role played by the central nervous system in the production of electrocardiographic abnormalities. As indicated in the case of acute cerebrovascular accidents and acute cerebral injury, these primarily involve disturbances of cardiac rhythm and repolarization. Investigations of this nature were already conducted more than fifty years ago indicating a distinct relationship between the central nervous system and cardiac electrical activity.³¹

As previously indicated, the sympathetic nervous system has rich central nervous representation. Intracranial stimulation of the sympathetic system may occur in the cortex, particularly in the motor and premotor regions, in the posterior hypothalamus, midbrain, pons, and medulla, and in the decussations between these areas. The area that appears to be most intimately linked with the cardiovascular system is the orbital surface of the frontal lobe (area 13), followed by the anterior cingulate gyrus (area 24). Area 13 is the main cortical representation of the parasympathetic nervous system and has a relay station in the anterior hypothalamus, with connections in the midbrain, pons, and medulla. The result of stimulation of this area is a rise or fall in blood pressure and alterations in cardiac rate. Thus, Kaada, observing the autonomic responses following stimulation of the ventral and medial aspects of the cerebral hemispheres, recorded inhibition of respiration, slow increase of blood pressure, or sudden drop of blood pressure upon stimulation of

the orbital surface of the frontal lobe.³¹

Vessels of the circle of Willis have an autonomic innervation from the glossopharyngeal and vagus nerves and from a continuation of the cervical sympathetic chain. Pool believed that stimuli applied to the circle of Willis were more likely to cause electrocardiographic abnormalities than when applied to cortical and cranial nerves other than the glossopharyngeal and vagus.³²

Berger, Liebowitz, and Herd showed that electrocardiographic abnormalities i.e., inverted T waves and depressed S-T segments, could be produced by infusions of epinephrine and norepinephrine into a coronary artery of an unanaesthetized dog in the absence of a rise in serum lactic dehydrogenase, suggesting that adrenergic reflexes can produce electrocardiographic changes without preceding myocardial necrosis.³³

Korteweg and co-workers studied the effects of stimulation of subcortical areas in cats, and found that stimulation of the basal ganglia resulted in immediate, albeit occasional, extrasystoles. Stimulation of the posterior hypothalamus and the quadrigeminal bodies produced ventricular extrasystoles, wandering pacemaker, and T wave changes. After stimulation had ceased, ventricular extrasystoles, atrioventricular dissociation, wandering pacemaker, tachycardia, and T wave changes were noted, all of which were abolished by vagal section.³⁴

More than 75 years ago, in 1930, Beattie, Brow, and Long produced extrasystoles by stimulation of the posterior hypothalamus in anaesthetized cats.³⁵ A destructive lesion of the posterior hypothalamus or section of the brain behind this level caused these extrasystoles to disappear. A study by other workers who subjected numerous points of the mid-brain of the cat to stimulation showed that hypo-

thalamic irritation resulted in distortions of the T wave and various arrhythmias.³⁶

Electrocardiographic changes following experimental craniocerebral injury -----

Jacobson and Danufsky, using albino Swiss mice, studied the effects of experimental craniocerebral injury in producing electrocardiographic changes.³⁷ Within 20 seconds of induction of head injury, the animals developed bradycardia, increased amplitude of the T wave, and a variety of arrhythmias- nodal rhythm, sinus arrhythmia, incomplete atrioventricular block, ventricular extrasystoles, and sinoatrial block. At autopsy, all the animals had subdural and subarachnoid haemorrhages while a few had cortical lacerations and one had an intracerebral haemorrhage.

Further experimental investigations by other workers involved embolism production by means of injection of paraffin oil into the carotid artery.³⁸ Shortly after injection of the paraffin, electrocardiographic changes consisting of bradycardia, prolonged Q-T interval, and deeply inverted T waves developed.

More recent neurological experiments have been carried out in an attempt to determine a relationship between the site of an acute cerebrovascular accident and the development of disturbances in cardiac rhythm, conduction and repolarization. The left insular cortex of the rat contains a site of cardiac chronotropic representation,³⁹ and prolonged phasic stimulation in this area results in atrioventricular block, prolongation of the Q-T interval, and ST segment depression.⁴⁰

Because the left insular cortex of the

rat contains a site of cardiac representation, Oppenheimer postulated that there might be a difference in cardiac expression between right- and left-sided lesions.¹⁶ There is evidence that asymmetries in brain function influence the heart through ipsilateral pathways, and there is a reported association between right hemispheric strokes and tachyarrhythmias of supraventricular origin and left hemispheric strokes and arrhythmias of ventricular origin.⁴¹ Other reports draw attention to the cardiovascular effects of human insular cortex stimulation,⁴² and there is a reported association between neurological intervention in the region of the left insular cortex and premature ventricular complexes with prolonged Q-T interval.⁴³

The cardiac morphological substrate accompanying intracranial lesions and their associated electrocardiographic abnormalities

Three reports dating back 38 years are of watershed significance with regard to the question of what morphological substrate may underlie electrocardiographic abnormalities occurring in association with intracranial lesions.⁴⁴⁻⁴⁶ Greenhoot and Reichenbach⁴⁴ and Hammermeister and Reichenbach⁴⁵ described the case of a 49-year-old woman with a subarachnoid haemorrhage. During her hospitalization, the patient initially had nonspecific ST segment changes on the electrocardiogram. However, five days after surgery, the electrocardiogram had changed into the pattern of a transmural infarction of the inferior and lateral walls of the left ventricle. Levels of serum enzymes, such as SGOT and LDH, and serum electrolytes were all within normal limits. Subsequent histopathological examination of the myocardium, however, revealed multiple foci of myo-

cyte necrosis, i.e., focal myocytolysis throughout the left ventricular myocardium.

Duren and Becker⁴⁶ also documented a clinico-pathological correlation between the development of the electrocardiographic pattern of a transmural myocardial infarction and focal myocytolysis of the myocardium. These workers described two patients, one with a cerebral infarct and one with a primary brain tumour, both of whom initially had a normal electrocardiogram but who subsequently developed the classic pattern of a transmural anteroseptal myocardial infarction. However, at autopsy, the naked-eye appearance of the myocardium did not present the changes expected in myocardial infarction. The discrepancy between massive transmural myocardial infarction clinically and electrocardiographically and the complete lack of corroborative and confirmative data from naked-eye inspection of the cut surface of the myocardium therefore suggested an underlying disease process different from that of classic myocardial infarction and it was only on subsequent histopathological examination that the features of focal myocytolysis were observed.

While in the first patient the active stage of focal myocytolysis with focal cardiomyocyte necrosis and mononuclear infiltration was noted, the second patient presented a problem in this regard. His electrocardiographic changes originated approximately four months before death. The clinical 'follow-up' suggested that at the time of death, a healing stage of the cardiac lesion had been reached and, indeed, subsequent histopathological examination of the myocardium disclosed 'healed lesions'. These latter lesions were mainly characterised by a fine meshwork of connective tissue fibres replacing lost myocardial cells. Such foci had to be differentiated from miliary infarcts, a distinction not always easy

to make. However, this case also exhibited minute areas in which actively degenerating myocardial cells were still identifiable. Furthermore, active lesions, as seen in the first patient, may eventually heal, leaving scars of a type seen in the second patient. In view of these findings and the fact that otherwise no classic myocardial infarction was present, these authors concluded that focal myocytolysis was also the underlying disorder in this latter patient as well. The microscopic features of healing were in keeping with and compatible with a time lapse of approximately four months.

Further confirmatory evidence of the role played by intracranial lesions in the production of electrocardiographic abnormalities in both adults and in the paediatric age group-----

More recent confirmatory evidence has emerged of the role played by intracranial lesions in the production of electrocardiographic abnormalities in both adults and children.⁴⁷⁻⁶² The finding of such ECG abnormalities in the paediatric age group is particularly significant in view of the fact that other than in congenital heart disease as an antecedent factor, the paediatric heart has, apart from rheumatic heart disease, lesser duration of exposure to predisposing factors in contrast to that operative in adults leading to ventricular hypertrophy, ventricular remodeling and cardiac failure.

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CHAPTER 7

MATERIALS AND METHODS

Forty two consecutive cases of fatal craniocerebral injury coming to medicolegal autopsy at the State Mortuary, Johannesburg over a six-month period were examined on a prospective basis after ethics clearance had been obtained from the Ethics Committee of the University of the Witwatersrand. In order to maintain patient confidentiality all tissue samples of myocardium taken at medicolegal autopsy were processed and identified solely by means of a number sequentially allocated thus preserving patient anonymity. While not a strict requirement in view of the fact that these constituted medicolegal samples taken in order to determine the pathological processes involved in death causation, nevertheless where the next-of-kin were known and could be traced verbal consent was obtained for taking 1 cm³ blocks of cardiac tissue, notwithstanding the fact that tissue sampling constitutes an integral part of a standard autopsy.

Other than the first two cases in the study who had survived for a brief 20-minute period after injury (survival period being defined as the time interval between injury and demise with this time period being determined by reference to police, scene and paramedic records accompanying the body to the mortuary together with a summary of the clinical records) all other cases had been admitted to and treated in the Department of Neurosurgery at the Johannesburg General Hospital. Patient management in this latter department enabled perusal of the clinical records in the department in conjunction with the then Head of Department Professor V. Farrell. In order to maintain patient confidentiality all records were perused in the department proper with no records being removed from the department and no indication given as to which patients

comprised those included in the study. Perusal of the clinical records thus not only permitted exclusion of any patients with a history of cardiac disease but also indicated that other than during the agonal period when desperate resuscitative measures had been performed, no catecholamine agents had been administered.

In each subject the following was documented: i) age, sex and ethnic group; ii) height, body weight and heart weight; iii) manner in which the injury had been sustained i.e., whether in a motor vehicle accident, a fall from a height, a gunshot wound, or blunt trauma to the head in the course of an assault, or in other miscellaneous manners; iv) whether any neurosurgical procedures had been performed and, if so, the nature of the procedure, and; v) the duration of patient survival following injury.

In order to facilitate statistical analysis, all traumatic craniocerebral lesions noted at autopsy were scored being graded on an increasing scale of depth and severity, an extension and modification of the scheme of contusion indices proposed by Hume Adams and co-workers.^{1,2} The reason for extension of this latter scheme in the present study is that while these workers applied an index to the depth and extent of contusions, the most frequent evidence of brain damage after head injury, they did not include a more comprehensive method of system analysis, similarly based on depth of lesion and type of lesion which must be taken into account in assessing the overall severity of a head injury. Thus, in the current 'modified' scheme, skull fractures, extradural haematomas, subdural haematomas, and subarachnoid haemorrhages were also scored in addition to contusion haemorrhages proper. A further differentiation and divergence from the scheme of contusion indices adopted by Hume Adams and his co-workers was that while these latter workers only graded depth of lesion into white matter making

no distinction being made between subcortical and deep white matter such distinction was taken into account in the present study. Depth of contusion haemorrhages is considered to be of paramount importance since the magnitude of vasogenic oedema is considerably greater in experimental animals with contusions that extend into the deep white matter compartment than in those with surface injuries.^{3,4} It is for this reason in addition to its severely disruptive effects on cortical tissue that a burst lobe was accorded the most severe score.

Craniocerebral lesions were thus graded and scored as follows (Chart I): a) skull fracture = 1 (Fig. I); b) extradural haematoma = 1 (Figs. II and III); c) subdural haematoma = 1 (Fig. IV); d) sub-arachnoid haemorrhage = 1 (Figs. V to VII); e) contusion haemorrhages, on an increasing scale of depth and severity, i.e.: i) grey matter of cortical ribbon = 1; ii) subcortical white matter = 2; iii) deep white matter/grey matter of basal ganglia/midbrain or pons = 3; iv) burst lobe with traumatic disruption of cortical tissue and extension of haemorrhage to the ventricular system or the overlying subarachnoid and/or subdural spaces = 4 (Figs. VIII to XV).

Chart I

Grading of Traumatic Craniocerebral Lesions (modified from scheme of Contusion Indices proposed by Hume Adams and co-workers)^{1, 2}

- a) Skull Fracture = 1;
 - b) Extradural Haematoma = 1;
 - c) Subdural Haematoma = 1;
 - d) Subarachnoid Haemorrhage = 1;
 - e) Contusion Haemorrhages, on an increasing scale of depth and severity, i.e.:
 - I. grey matter of cortical ribbon = 1;
 - II. subcortical white matter = 2;
 - III. deep white matter/grey matter of basal ganglia/midbrain/pons = 3;
 - IV. burst lobe with traumatic disruption of cortical tissue and extension of haemorrhage to the ventricular system or the overlying subarachnoid and /or subdural spaces = 4.
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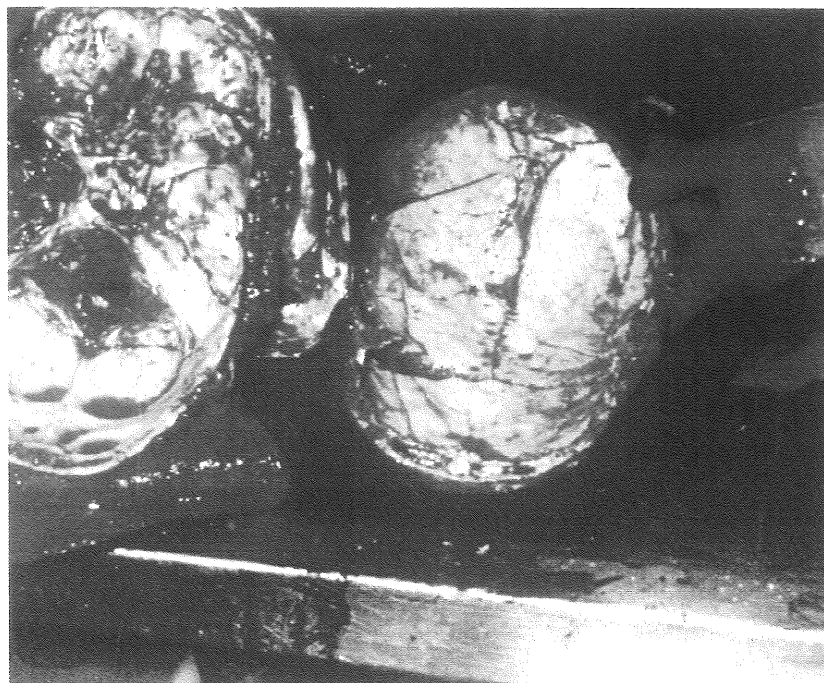


Fig. I. Multiple comminuted and linear fractures of the base of the skull extending to involve the calvarium.

Case no. 1; Craniocerebral score = 6

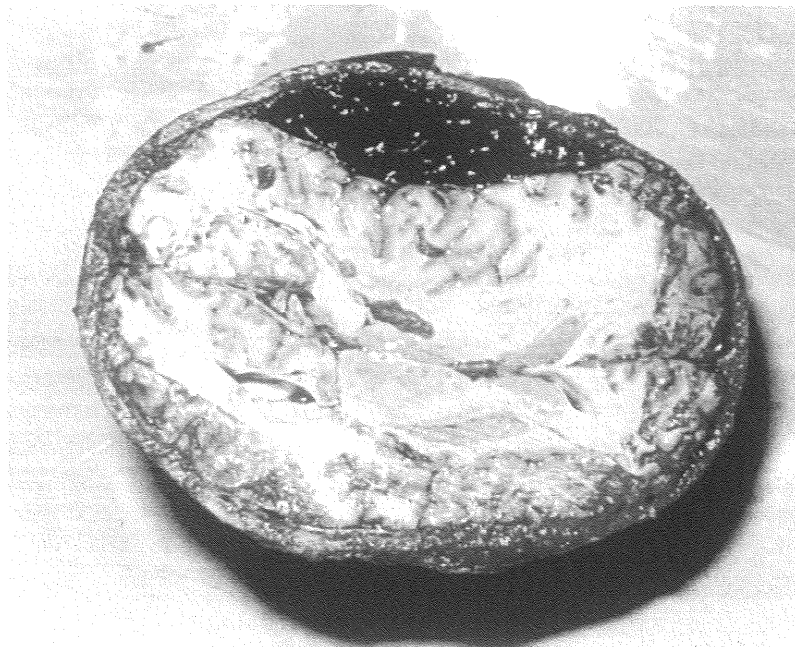


Fig. II. An extradural haematoma showing compression of the underlying brain with midline shift.

Case no. 15; Craniocerebral score = 3.

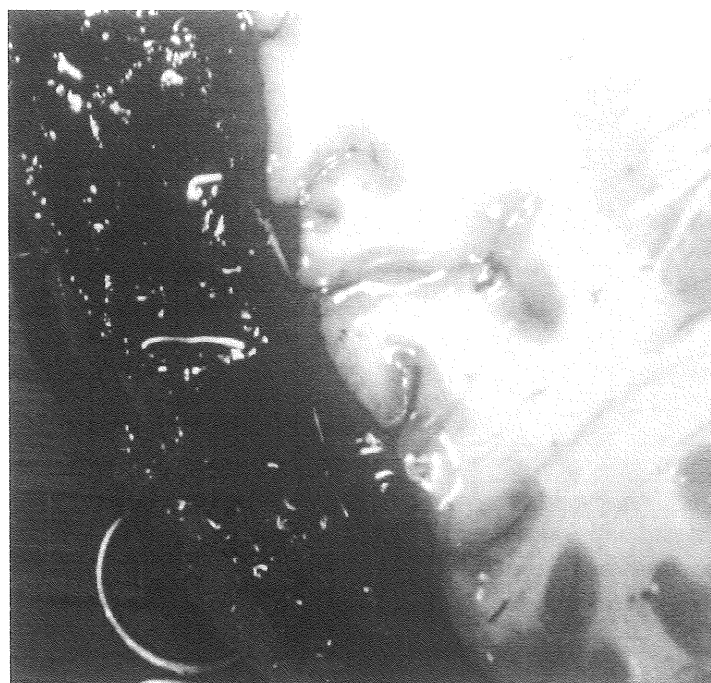


Fig. III. A higher magnification of the extradural haematoma depicted in Figure II showing compression of the underlying brain in which oedema of the grey matter of the cortical band and the underlying white matter is noted.

Case no. 15; Craniocerebral score =3.

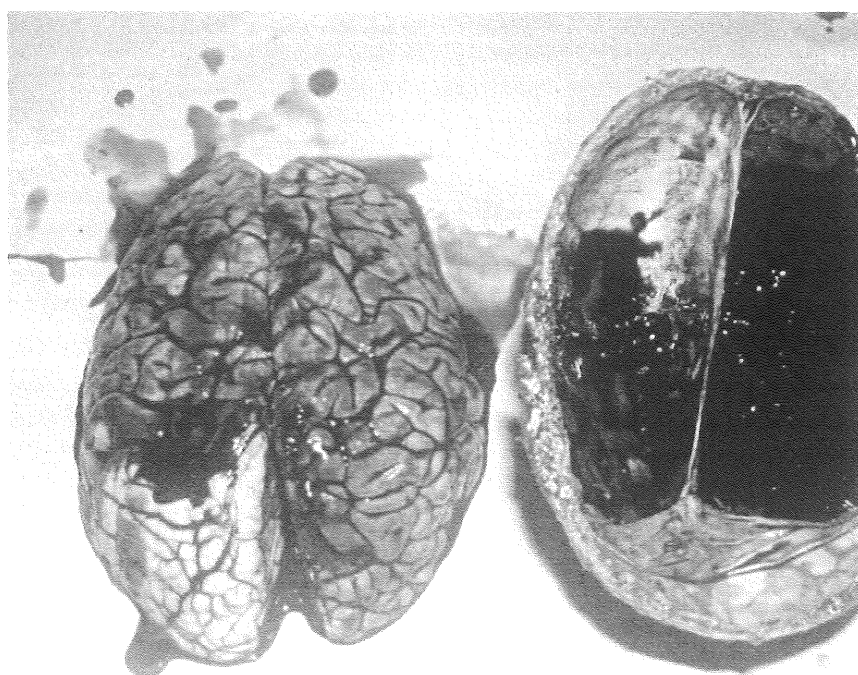


Fig. IV. A subdural haematoma seen beneath the dura over the inner aspect of the calvarium with remnant of a smaller haematoma seen over the lateral aspect of one temporo-parietal area.

Case no. 9; Craniocerebral score =4.



Fig. V. Multiple focal subarachnoid haemorrhages seen over the base of the brain and involving the ventral cerebellum, the ventral brainstem and both temporal lobes inferiorly and laterally.

Case no. 20; Craniocerebral score =4

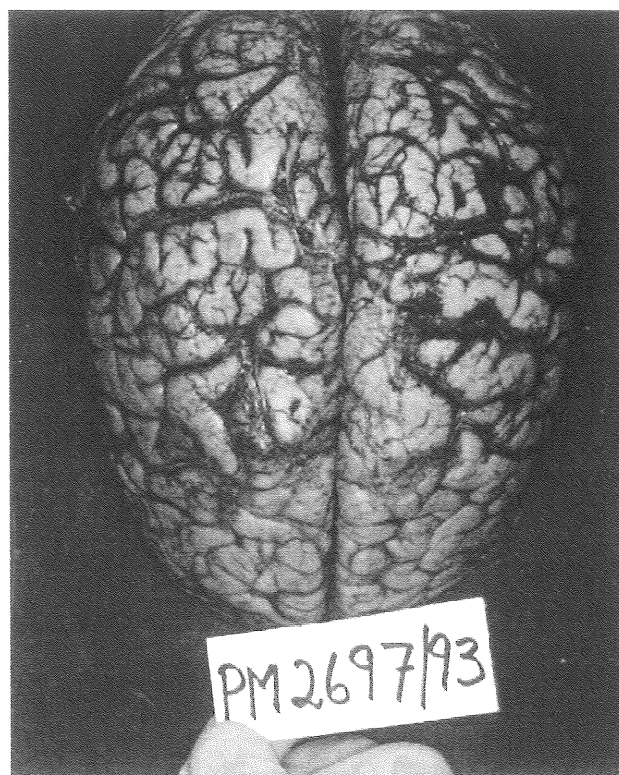


Fig. VI. Multiple thin-layered subarachnoid haemorrhages predominantly involving the left parietal and left occipital lobes of the left cerebral hemisphere over its lateral and supero-lateral aspects.

Case no. 25; Craniocerebral score =3

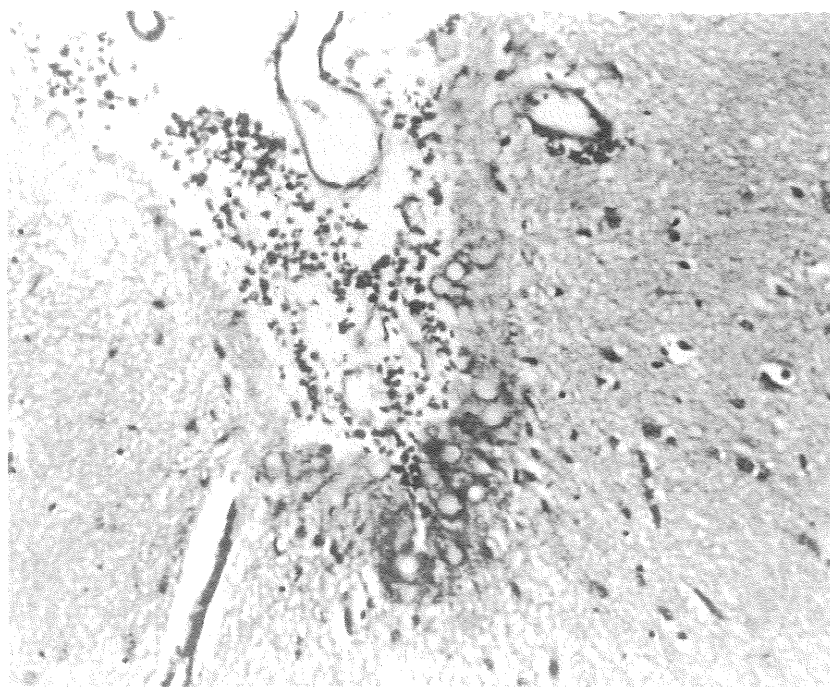


Fig. VII. Subarachnoid haemorrhage extending into the depths of a sulcus (Haematoxylin and eosin x 400).

Case no. 25; Craniocerebral score =3

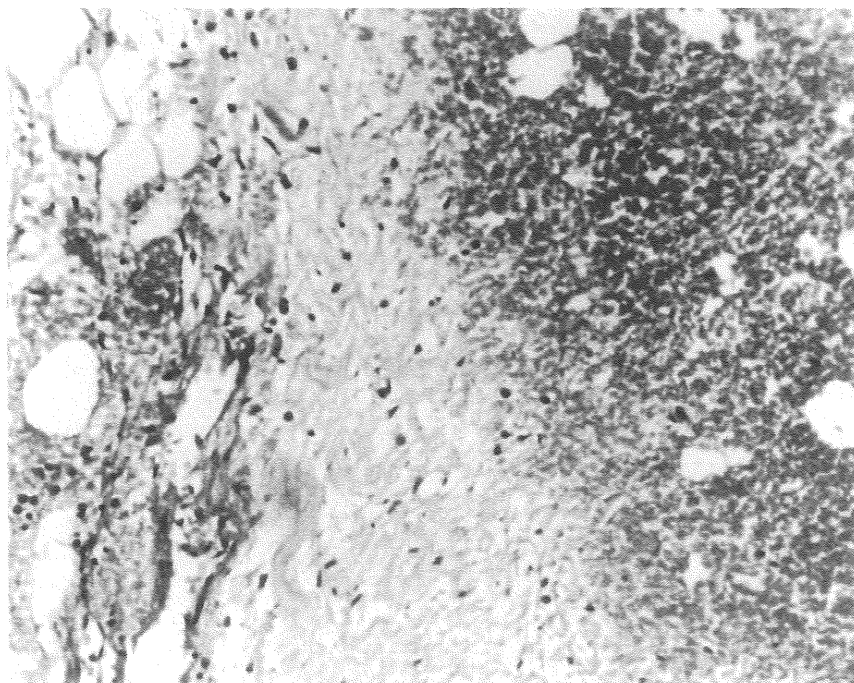


Fig VIII. A contusion haemorrhage involving the grey matter of the cortical ribbon and merging into the leptomeninges (pia and arachnoid) (Haematoxylin and eosin x 400).

Case no. 17; Craniocerebral score =5.

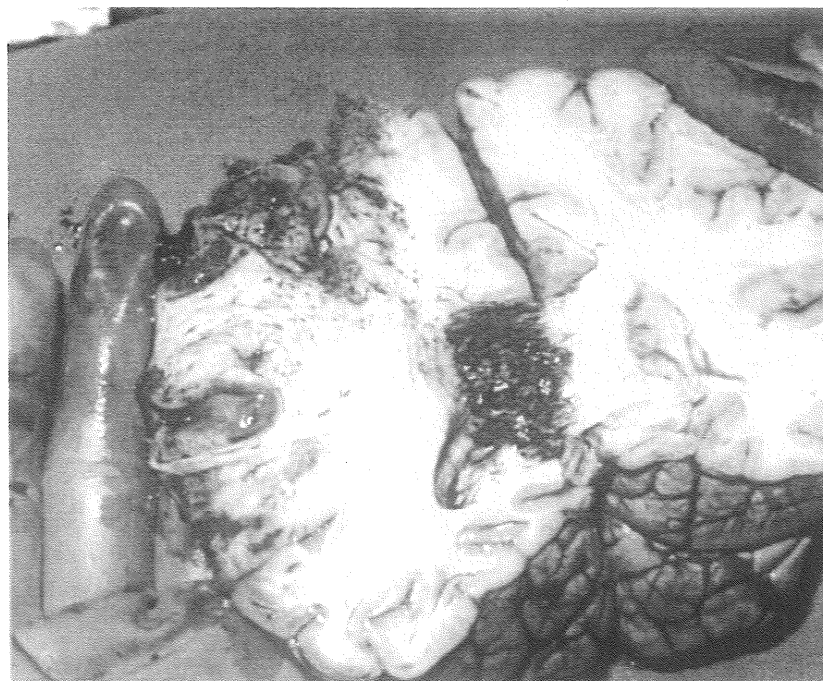


Fig. IX. A coronal section of the brain showing cortical contusional haemorrhage involving the grey matter of the cortical ribbon as well as the subcortical white matter and deeper white matter

Case no. 13; Craniocerebral score =8

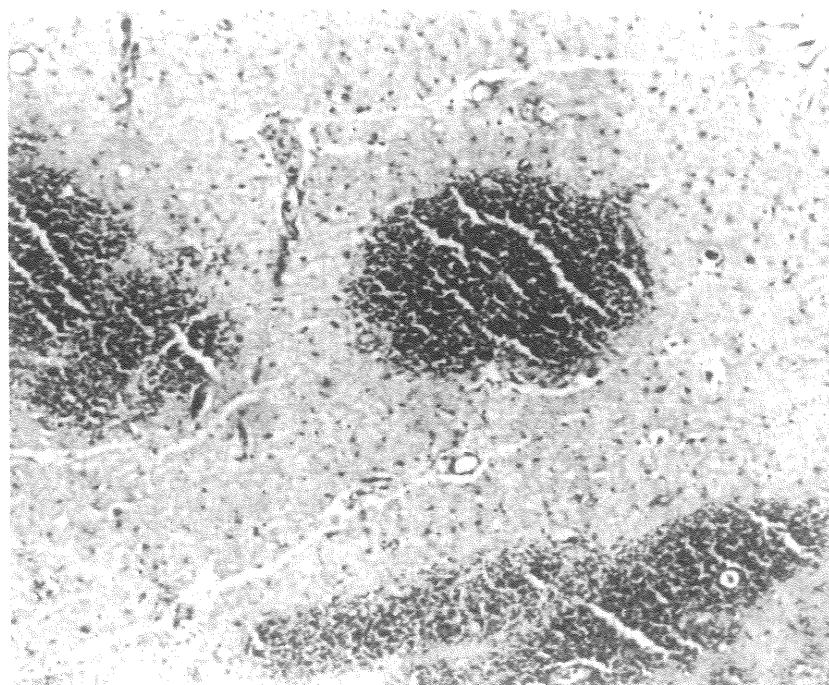


Fig. X. Multiple contusional haemorrhages involving the subcortical white matter (Haematoxylin and eosin x 400).

Case no. 21; Craniocerebral score =4.

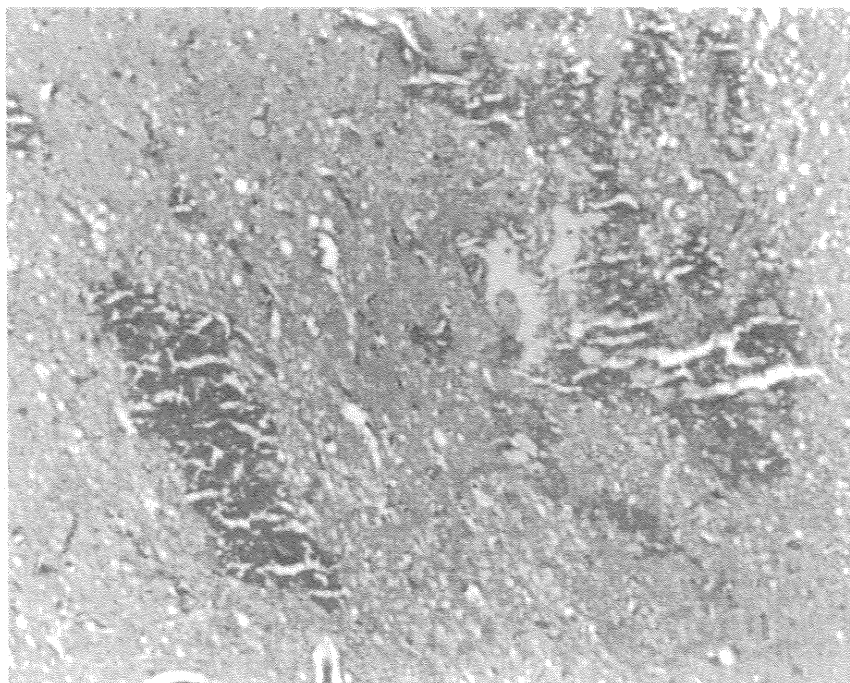


Fig. XI. Multiple contusional haemorrhages involving the deep white matter. The surrounding neuropil shows spongiosis indicative of cerebral oedema (Haematoxylin and eosin x 400).

Case no. 22; Craniocerebral score =9.

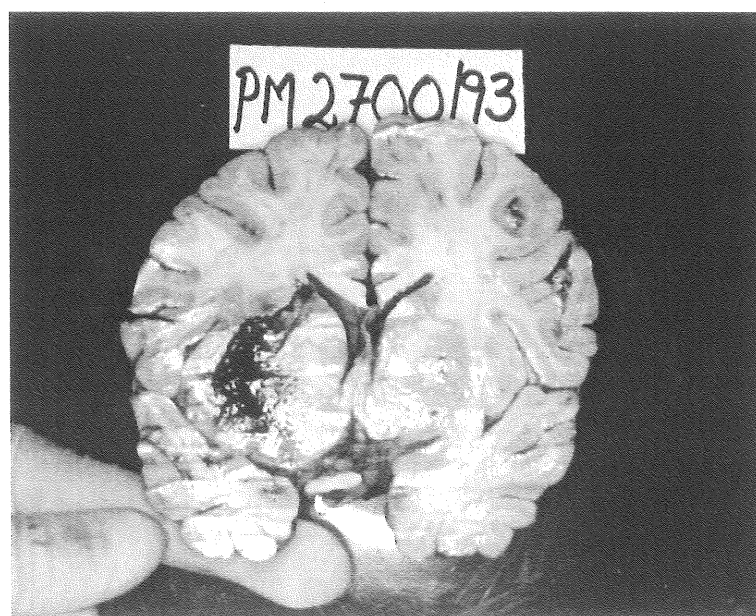


Fig. XII. A coronal section of the brain showing contusional haemorrhage involving the deep white matter and extending medially to the basal ganglia.

Case no. 26; Craniocerebral score =4.



Fig. XIII. A coronal section of the brain showing a burst parietal lobe with the rupture extending from the deep white matter to the overlying subarachnoid and subdural spaces.

Case no.18; Craniocerebral score =8

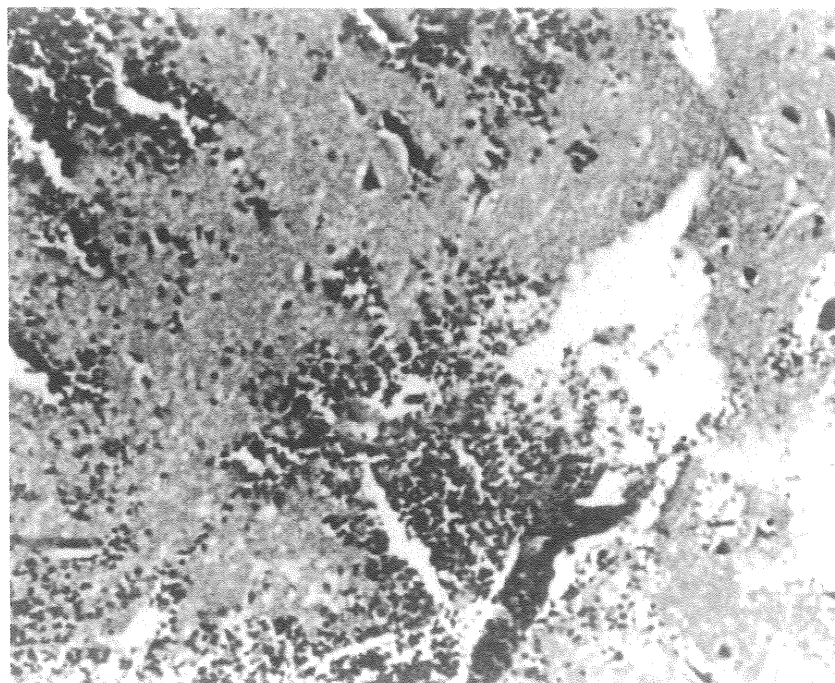


Fig. XIV. A burst lobe with haemorrhage dissecting through and upwards towards the cortical surface (Haematoxylin and eosin x 180).

Case no. 6; Craniocerebral score =8

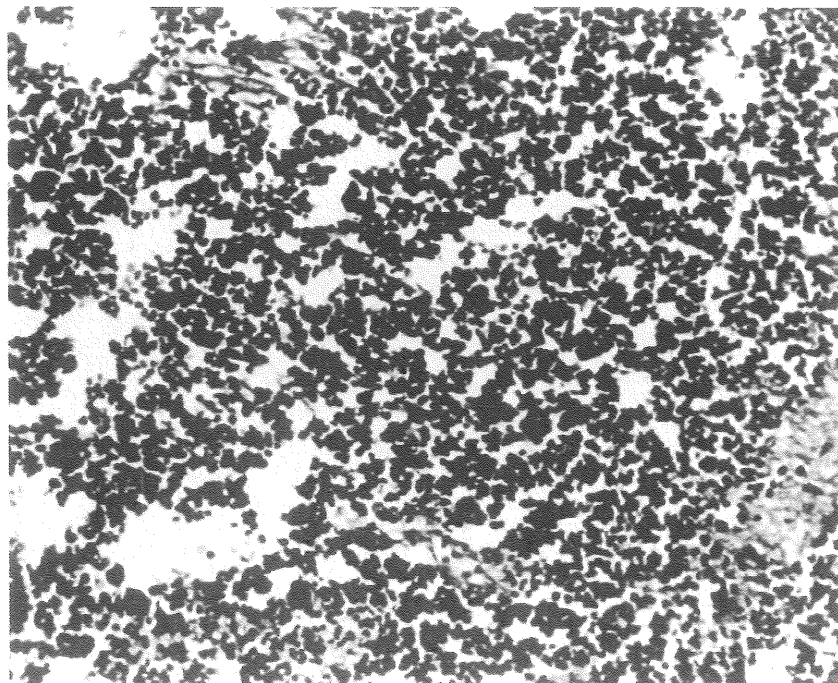


Fig. XV. A burst lobe showing the extensive nature of the haemorrhage dissecting through and disrupting the cortical tissue (Haematoxylin and eosin x 160).

Case no. 16; Craniocerebral score =9

In view of the fact that cardiac histopathological changes may be both focal but nevertheless global in distribution, all cardiac specimens taken at autopsy were fixed in 10% neutral buffered formalin and examined in accordance with the technique and criteria of cardiac mapping devised by Olsen.⁵ This methodology was specifically developed by this worker in order to determine the presence of global cardiac pathology and is therefore particularly suitable for delineating what may be patchy and focally distributed areas of histopathological change. With regard to the coronary arteries, examination was carried out by means of transverse incisions at 3 to 4mm intervals throughout the major coronary arteries. Individual transverse incisions were then sequentially linked by longitudinal incisions (cuts) with fine scissors. This latter methodology can and should be performed in order to provide data adequate for clinico-pathological correlation.⁶

Olsen's method thus comprised the taking of tissue blocks from 20 separate and specific areas of myocardium, as follows:-

i) blocks a-h comprised sequential clockwise sections taken through the total circumference of the left ventricle 3cm proximal to the apex;

ii) blocks i-k comprised sequential clockwise sections taken through the circumference of the right ventricle at the same transverse level as the left;

iii) blocks l and m comprised sections taken through the posterior papillary muscle complex;

iv) blocks n and o comprised sections taken through the apices of the left and right ventricles respectively;

v) block p comprised a section taken through the right ventricular outflow tract;

vi) block q comprised a section taken through the left atrial appendage;

vii) block r comprised a section taken

through the posterior wall of the left atrium;

viii) block s comprised a section taken through the right atrial appendage;

ix) block t comprised a section taken through the posterior wall of the right atrium.

The blocks thus taken from the above areas were then processed and embedded for paraffin sectioning and cut at 5 μ thickness. Eight hundred and forty blocks were hence obtained. A minimum of three serial sections per block were stained with haematoxylin-eosin, Masson's trichrome, elastic-van Gieson and phosphotungstic-acid haematoxylin (PTAH) yielding a total of 10,080 sections. The effort involved in scoring so many sections was in accordance with the basic fundamentals of statistical practice and methodologies in that the greater number of sections examined the more valid will be the statistical conclusions drawn.

These blocks were then examined by conventional light microscopy by myself in conjunction with a Principal Specialist in histopathology (Anatomical Pathology) from the department of Anatomical Pathology of the Faculty of Health Sciences at the University of the Witwatersrand and holder of an FRC Path. in order to provide standardisation and to eliminate observer error. This latter pathologist similarly assisted in selection of histopathological slides for illustration purposes and lesion demonstration.

Quantification of histological changes with scoring according to severity and extent

All histopathological changes noted were quantified according to severity on the following basis, with a score being allocated to each finding.⁷⁻¹⁹

I. Nature of histopathological change and severity

a) Interstitial oedema, myocardial fibre vacuolation, cloudy swelling and smudging of fibres

1+ = presence of a mild degree of interstitial

oedema with solitary cells showing myocardial fibre vacuolation / cloudy swelling and smudging of fibres;

2+ = presence of a moderate degree of interstitial oedema with foci of several cells showing myocardial fibre vacuolation / cloudy swelling and smudging of fibres;

3+ = presence of a severe degree of interstitial oedema with a large number of cellular foci showing myocardial fibre vacuolation / cloudy swelling and smudging of fibres.

b) Focal myocytolysis

1+ = presence of solitary cells showing focal myocytolysis;

2+ = presence of several cellular foci of focal myocytolysis;

3+ = presence of a large number of foci of focal myocytolysis.

c) Mononuclear infiltration and proliferation

1+ = presence of solitary cells around vessels or between muscle fibres;

2+ = presence of clusters of cells around vessels or between muscle fibres;

3+ = diffuse infiltration of the entire area of myocardium examined.

d) Interstitial fibrosis

1+ = presence of loose granulation tissue in interstitial spaces, around vessels or foci of such tissue i.e., evidence of stromal condensation;

2+ = presence of small foci of dense collagenous tissue;

3+ = numerous foci of collagenous tissue scattered throughout the myocardium.

e) Nuclear changes

1+ = enlargement and vesiculation of nucleus with prominence of nucleolus;

2+ = brick-shaped blunt-ended nuclei with peripheral margination of chromatin together with the foregoing changes of 1+ above;

3+ = crenation of nuclear membrane.

II. Grading according to extent of histological changes noted within a high-power field (HPF)

1+ = changes (as defined above) noted within 1 - 2 HPF;

2+ = changes noted within 3 - 4 HPF;

3+ = changes noted within 5 - 6 HPF;

4+ = changes noted within 7 - 8 HPF;

5+ = changes noted within 9 -10 HPF.

The allocation of scores in this manner to the various histopathological changes observed in the hearts of previously head-injured victims thus constitutes an attempt to scientifically quantify these changes and render them in statistical terms. Hence, the scoring system thus derived with separation of the heart into 20 different areas of myocardium examined together with allocation of a score to the frequency of involvement of each area of the heart according to the extent and severity of the type of pathological change encountered in each area enabled statistical application of the Mann-Whitney U-test for statistical evaluation.

Statistical formulation and evaluation

In this study, an attempt was made to determine whether any statistically significant relationship existed bet-

ween craniocerebral injury on the one hand and the different histopathological variables interstitial oedema, focal myocytolysis, mononuclear infiltration, fibrosis and nuclear changes on the other. To this end, two questions were essentially formulated in this exploratory study and subsequently subjected to statistical reduction and analysis, as follows:-

1. Is there a myocardial change related to a scored craniocerebral injury that may be a predictor for survival outcomes in transplanted cardiac patients?, and;
2. Is there a predictable significant change that occurs in the myocardium following a certain craniocerebral injury score?

In this regard, statistical analysis was performed by a biostatistician from the Institute of Biostatistics of the Medical Research Council (MRC) and holder of a Ph.D. degree in biostatistics.

The Mann-Whitney U-test was applied to the variables age, gender, ethnic group, type of myocardial histopathological lesion and the total scores of these histopathological lesions, the latter a summation of the individual scores obtained in each case. The Mann-Whitney test is applied where the observer draws two independent samples, for example age and ethnic group, from the same population and compares the two on a single criterion. Hence, descriptive statistics were performed on all these variables and comparisons made between certain variables. As the Mann-Whitney test assumes data to be continuously distributed, it could be applied to the variable craniocerebral injury as the values here ranged on a continuous scale increasing from one to nine. Age, too, was a continuous variable while the variables ethnic group and gender were used as categorical data and the Mann-Whitney test similarly applied. This test was also applied in the case of the histopathological variables focal myocytolysis, mononuclear infiltration, and fibrosis.

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CHAPTER 8

RESULTS^{*}

Gender, ethnic group, and age distribution of subjects

Of the 42 subjects examined, 35 (83.3%) were male and 7 (16.7%) female. Thirty subjects were black (71.4%), 11 were white (26.2%) and 1 (2.4%) an Indian subcontinent Asaian (Table 1). The subjects ranged in age from 3 to 70 years, with the greatest number of subjects 11 (26.2%) falling into the 21 to 30 year age group paralleling the distribution seen in studies of traumatic injury to other organ systems conducted by the author of the current study.^{1,2} In descending order of frequency, the remaining age distribution was as follows:- 11-20 years, 9 subjects (21.4%); 41-50 years, 7 subjects (16.7%); 31-40 years, 5 subjects (11.9%); 0-10 years, 4 subjects (9.5%); 51-60 years and 61-70 years, 3 subjects each (7.1%).

Manner and incidence of injury

Twenty four (57.1%) subjects had sustained fatal cranio-cerebral injury in the course of a motor vehicle accident, 13 (31.0%) in an assault, 3 (7.1%) in miscellaneous traumatic manners, and 2 (4.8%) in falls from a height.

^{*} This work was presented under the title 'The Potential Long-Term Cardiac Implications of Antecedent Cranio-cerebral Injury' at the 15th Triennial Meeting of the International Association of Forensic Sciences held at UCLA, California, over 22-28 August, 1999 and was published in abstract form in the Proceedings of the IAFS, 1999: 196 and in Cardiovascular Pathobiology 1998; 2 (3-4): 207-218.

However, none of the subjects had sustained direct thoracic trauma or cardiac contusion. Neurosurgical procedures had been performed in 8 (19.0%) of the 42 subjects, the procedure in each case being a craniotomy with evacuation of either an extradural or subdural haematoma.

Microscopic examination and histopathological findings

The earliest light microscopic structural changes noted were interstitial oedema, myocardial fibre vacuolation, and alterations in nuclear morphology. These changes were evident in a wide range of subjects of differing ages, genders, ethnic groups and survival periods including subjects with only 20 minutes of post-injury survival (cases 1 and 2) (Table 1).

Interstitial oedema

Interstitial oedema manifested as marked separation of myocardial fibres throughout the heart with prominence and exaggeration of intercellular spaces (Fig. 8.1; case no. 1: 20 minutes of post-injury survival). Intracellularly, a fine microvacuolization was noted. While initially interpreted as postmortem change, this microvacuolization was present even when there had been no prolonged agonal period and the interval between death and postmortem fixation was short.

Interstitial oedema was present in all cases in all 20 areas of the myocardium examined and persisted until day 27 after injury (case no. 37) following which it rapidly decreased in extent from involving 100 per cent of the areas examined to 30 per cent. It persisted in these latter areas until day 33 after injury (case no. 39), following which it apparently resolved, disappearing entirely beyond this period in the sections examined (Table 2).

Table 1
POST-TRAUMATIC SURVIVAL PERIOD

Case no.	Age	Sex	Ethnic group	Height	Weight	Heart weight	Survival period
1.	32	M	Black	1.66 m	58 kg	410 g	20 minutes
2.	3	M	White	0.80 m	15 kg	65 g	20 minutes
3.	20	M	Black	1.66 m	65 kg	350 g	2 hours
4.	5	F	Black	0.96 m	25 kg	105 g	3 hours
5.	21	M	Black	1.75 m	54 kg	310 g	7 hours
6.	23	M	Black	1.81 m	68 kg	310 g	9 hours
7.	16	M	Black	1.68 m	63 kg	295 g	15 hours, 30 mins
8.	46	F	White	1.74 m	94 kg	370 g	16 hours
9.	8	M	White	1.36 m	35 kg	135 g	20 hours
10.	40	M	Black	1.70 m	63 kg	320 g	21 hours
11.	45	M	Black	1.83 m	61 kg	385 g	29 hours
12.	11	M	Black	1.32 m	26 kg	185 g	30 hours
13.	43	M	Black	1.74 m	54 kg	270 g	32 hours
14.	26	M	Black	1.77 m	58 kg	320 g	36 hours
15.	19	M	White	1.76 m	66 kg	360 g	36 hours
16.	50	M	Black	1.64 m	65 kg	370 g	48 hours
17.	24	M	Asian	1.76 m	61 kg	260 g	60 hours
18.	26	M	White	1.69 m	47 kg	280 g	66 hours
19.	70	M	Black	1.66 m	49 kg	220 g	4 days, 12 hours
20.	50	M	White	1.64 m	68 kg	320 g	4 days, 12 hours
21.	19	M	Black	1.72 m	54 kg	290 g	4 days, 16 hours
22.	18	M	White	1.71 m	57 kg	310 g	5 days
23.	35	F	Black	1.66 m	57 kg	290 g	5 days
24.	54	M	Black	1.70 m	63 kg	380 g	5 days
25.	25	F	Black	1.62 m	51 kg	205 g	5 days, 2 hours
26.	54	M	Black	1.70 m	65 kg	360 g	6 days
27.	45	F	Black	1.67 m	55 kg	285 g	8 days, 4 hours
28.	22	F	White	1.76 m	48 kg	290 g	8 days, 15 hours
29.	65	M	White	1.79 m	78 kg	615 g	10 days
30.	20	M	Black	1.63 m	62 kg	230 g	11 days, 6 hours
31.	23	M	Black	1.71 m	52 kg	290 g	12 days
32.	15	M	White	1.74 m	84 kg	360 g	12 days, 2 hours
33.	42	M	Black	1.62 m	42 kg	370 g	13 days
34.	6	F	Black	1.15 m	34 kg	145 g	15 days
35.	32	M	Black	1.63 m	53 kg	310 g	22 days
36.	59	M	Black	1.72 m	60 kg	340 g	24 days, 10 hours
37.	35	M	Black	1.60 m	52 kg	375 g	27 days
38.	24	M	Black	1.78 m	47 kg	270 g	28 days
39.	22	M	White	1.72 m	43 kg	350 g	33 days
40.	28	M	Black	1.74 m	55 kg	390 g	36 days
41.	20	M	Black	1.89 m	40 kg	315 g	49 days, 16 hours
42.	66	M	Black	1.71 m	57 kg	250 g	5 months, 15 days

Footnote: Survival period is defined as the time interval between injury and subsequent demise, expressed in chronological terms.

Table 2

Scoring according to number of areas of myocardium involved by each type of pathological change.

No. of anatomical areas of myocardium involved per type of change (n = 20)											
Case	Age	Sex	Race	Survival period	CC injury/ score	Interstitial oedema	Necrosis	Mononuclear infiltration	Interstitial fibrosis	Nuclear alteration	Total score
1.	32	M	B	0.014	6	20	0	0	0	20	40
2.	3	M	W	0.014	2	20	0	0	0	20	40
3.	20	M	B	0.083	9	20	0	0	0	20	40
4.	5	F	B	0.125	2	20	0	0	0	20	40
5.	21	M	B	0.292	9	20	0	0	0	20	40
6.	23	M	B	0.375	8	20	0	0	0	20	40
7.	16	M	B	0.646	9	20	0	0	0	20	40
8.	46	F	W	0.667	1	20	0	0	0	20	40
9.	8	M	W	0.833	4	20	0	0	0	20	40
10.	40	M	B	0.875	9	20	0	0	0	20	40
11.	45	M	B	1.208	9	20	0	0	0	20	40
12.	11	M	B	1.250	1	20	0	0	0	20	40
13.	43	M	B	1.333	8	20	0	0	0	20	40
14.	26	M	B	1.500	7	20	0	0	0	20	40
15.	19	M	W	1.500	3	20	0	0	0	20	40
16.	50	M	B	2.000	9	20	0	0	0	20	40
17.	24	M	A	2.500	5	20	0	0	0	20	40
18.	26	M	W	2.750	8	20	8	8	0	20	56
19.	70	M	B	4.500	9	20	0	0	0	20	40
20.	50	M	W	4.500	4	20	5	5	0	20	50
21.	19	M	B	4.667	4	20	2	2	0	20	44
22.	18	M	W	5.000	9	20	12	12	0	20	64
23.	35	F	B	5.000	9	20	1	1	0	20	42
24.	54	M	B	5.000	2	20	5	5	0	20	50
25.	25	F	B	5.083	3	20	5	5	0	20	50
26.	54	M	B	6.000	4	20	5	5	0	20	50
27.	45	F	B	8.167	2	20	6	6	0	20	52
28.	22	F	W	8.625	2	20	10	10	0	20	60
29.	65	M	W	10.000	2	20	2	2	0	20	44
30.	20	M	B	11.250	2	20	4	4	0	20	48
31.	23	M	B	12.000	3	20	5	5	0	20	50
32.	15	M	W	12.083	9	20	11	11	0	20	62
33.	42	M	B	13.000	1	20	0	0	5	20	45
34.	6	F	B	15.000	9	20	0	0	3	20	43
35.	32	M	B	22.000	5	20	0	0	4	20	24
36.	59	M	B	24.417	1	20	0	0	3	20	23
37.	35	M	B	27.000	4	20	0	0	11	20	51
38.	24	M	B	28.000	6	6	0	0	13	20	39
39.	22	M	W	33.000	1	7	0	0	7	20	34
40.	28	M	B	36.000	9	0	0	0	14	20	34
41.	20	M	B	49.667	1	0	0	0	11	20	31
42.	66	M	B	167.000	3	0	0	0	6	0	6

CC = craniocerebral injury.

Footnote: The survival period is here expressed in statistical terms in percentages of 24 hour periods with simple arithmetical progression.

Total score refers to total no. of areas of myocardium involved by a particular pathological lesion, not CC injury score, which is a separate value; this latter could not be related to outcome or carry prognostic significance.

Concomitant with the above changes, a number of distinct patterns of myocardial cellular injury began to emerge. The earliest of these changes, vacuolar degeneration, appeared within 20 minutes of injury manifesting diffusely throughout the myocardium in the sections studied. The later changes myofibrillar fragmentation and cellular dropout, precursors of focal myocytolysis, followed a different pattern of myocardial involvement. While focal, they were nevertheless widely distributed throughout the myocardium and were sufficiently distinctive that once recognised were easily relocated on review of the sections or clearly seen on initial examination of new sections.

Vacuolar degeneration

The myocardial fibres developed vacuoles of varying size and concentration. While there appeared to be no uniform pattern of vacuole localisation, the process appeared to begin in the juxtannuclear zone. A vacuole, larger and seemingly more prominent than the nucleus, could be found on either side of the nucleus compressing and distorting it. In transverse sections, cells with this type of change exhibited a large empty hole surrounded by a rim of myofibril-poor cytoplasm of varying width i.e., manifesting a 'doughnut-like' appearance, a term originally coined by Grinvalsky and Fitch in describing a distinctive cardiomyopathy occurring in Omaha, Nebraska.³ Alternatively, multiple smaller vacuoles could be found diffusely throughout a myocardial fibre giving it an eroded and 'moth-eaten' appearance. In areas, these smaller vacuoles appeared to coalesce to form larger, more prominent vacuoles (Fig. 8.1).

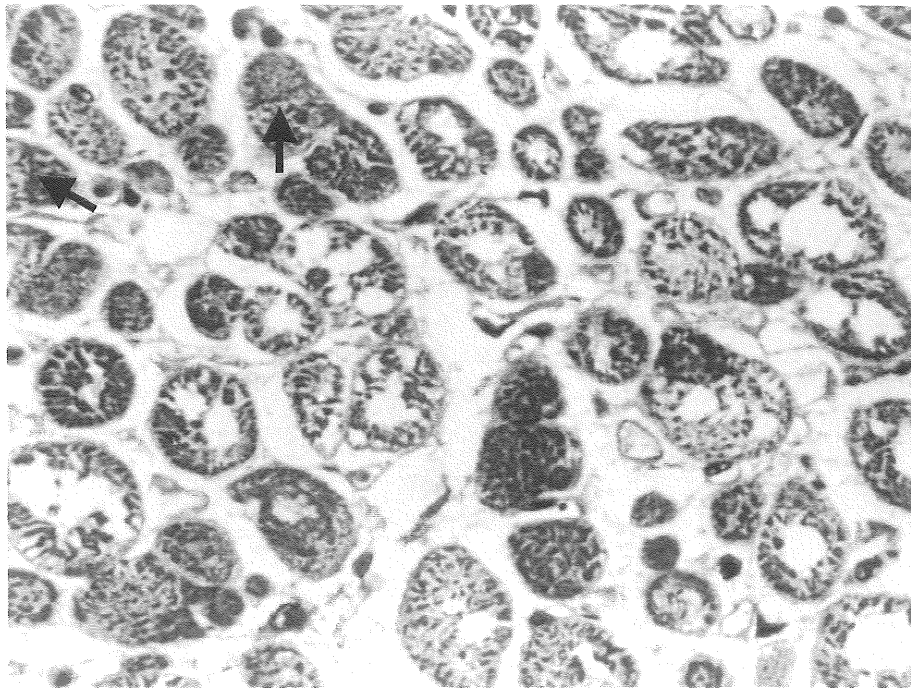


Fig. 8.1. Marked separation of myocardial fibres with exaggeration of intercellular spaces by interstitial oedema. Vacuoles of varying size are present within numerous myocardial fibres. In addition, contraction band necrosis is evident with opaque transverse band spanning the myofibres (arrows), distortion of myocardial fibres and aggregation of contractile material into thicker darker masses resembling 'Chinese writing'. Case no.1; 20 minutes of post injury survival

(Haematoxylin and eosin x 80).

Myofibrillar fragmentation

This appeared to involve the cardiomyocyte more uniformly than vacuolar degeneration. Myofibrils fragmented and disappeared throughout the cell in diffuse fashion while the residual cytoplasm showed a fine dust-like evenly dispersed granularity (Fig. 8.2; case no. 16: 48 hours of post-injury survival). While the process may begin in one or two cells it appeared with temporal progression to involve small clusters of cells. In transverse sections, small gaps caused by myocardial disintegration punctuated and interrupted the myocardial syncytium, imparting looseness and a seeming lack of cohesion to the myocardial architecture (Fig. 8.2). These changes, while focal, nevertheless occurred throughout the myocardium.

Alterations in nuclear morphology (nuclear change)

A wide spectrum of striking nuclear alterations were seen in all 20 areas of myocardium examined and being present in all cases of post-injury survival ranging from 20 minutes to 49 days (cases 1 to 41) (Table 2). Nuclei were enlarged, irregular and distorted. A particularly distinctive change was central clearing of the nucleus with peripheral margination and clumping of chromatin along an undulating nuclear membrane. Nuclei thus appeared brick-shaped, blunt-ended and vesicular (Fig. 8.2). Figures 8.1 and 8.2 (cases 1 and 16, respectively) manifested, in addition, the presence of contraction band necrosis. This appeared as opaque transverse bands with break up and distortion of the myocardial fibres and aggregation of contractile material into thicker darker masses having the appearance of 'Chinese writing'.

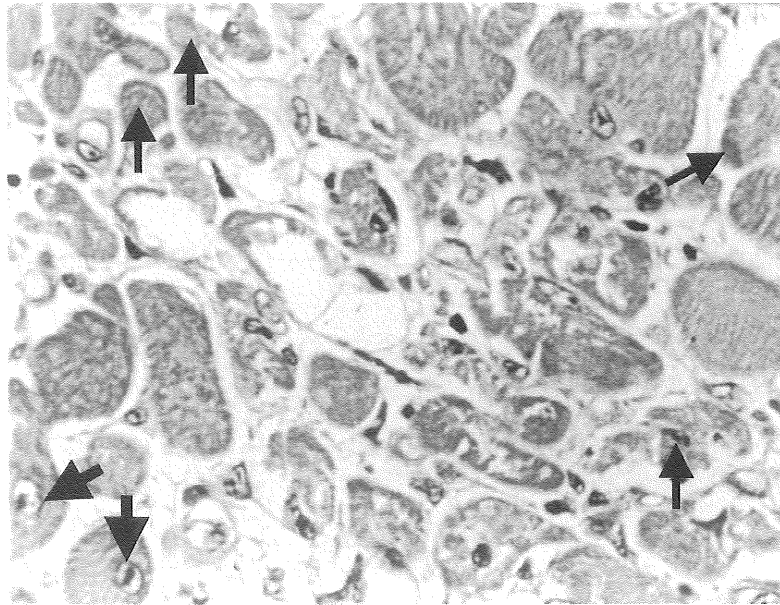


Fig. 8.2. An early focus of myocardial cellular disintegration in the centre of the field. Nuclear enlargement with vesiculation is noted in the left hand bottom of the field together with peripheral margination of chromatin (arrows). In addition, contraction band necrosis with aggregation of contractile material into 'Chinese writing' pattern is evident (thin arrows). Case no. 16; 48 hours of post-injury survival (Haematoxylin and eosin x 400).

While the time of appearance of the nuclear changes paralleled that of interstitial oedema it persisted longer. Unlike oedema, however, there was no tailing off and gradual decline of the process but rather an abrupt cessation at seven weeks (case no. 41) (Table 2). However, in view of the fact that no cases in the study survived beyond this period with the exception of a single case at five-and-a-half months, it is possible that these nuclear changes persist longer than the time observed in the study.

Focal myocytolysis

With progression to 66 hours after injury, a lesion showing a central area of myocardial cellular disintegration with partial collapse of the interstitial stroma and indistinct peripheral extension of the myocytolytic process into the surrounding tissues became manifest (Fig. 8.3; case no. 18: 66 hours of post-injury survival). At this time the lesion appeared in its earliest stages whether involving focal lysis of individual cardiomyocytes or small clusters of cells. With further temporal progression to 108 hours or more after injury and with more severe involvement, fascicular aggregates of cells displayed the change (Figs. 8.4 and 8.5; cases 20 and 21; 4 days and 12 hours and 4 days and 16 hours after injury, respectively).

With further progression of the process, a full-blown florid lesion became manifest. In contrast to the lesion seen at an earlier stage of evolution, the full-blown florid lesion was both more distinct and, despite its tongues of disintegrating tissue radiating and extending peripherally, more clearly demarcated from the surrounding myocardium. This lesion was present in all cases with five days or more of post-injury survival (cases 22 to 32; Figs. 8.6 to 8.8. (Table 1)).

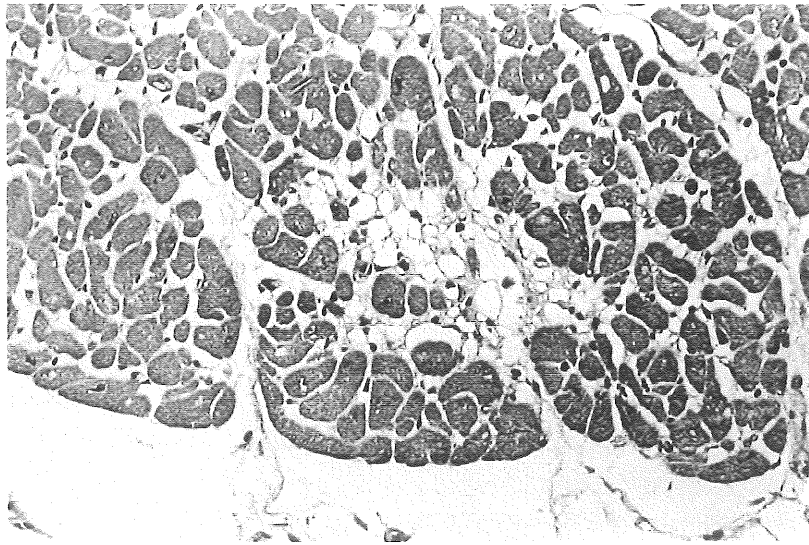


Fig. 8.3. An early but well-defined focus of cardiac myocytolysis with cellular dropout and showing some peripheral extension of the process in to the surrounding myocardium. Case no 18; 66 hours of post-injury survival (Haematoxylin and eosin x 160).

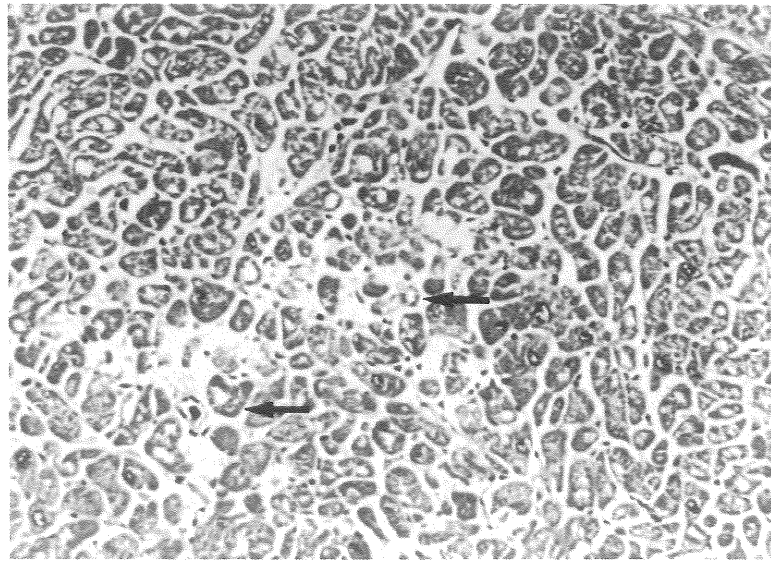


Fig. 8.4. A further stage in lesion evolution and progression. Cellular disintegration is present with peripheral extension of the process into the surrounding myocardium which shows a more florid mononuclear infiltrate than that within the lesion proper in which the infiltrate is noted by its paucity. Case no. 20; 4 days 12 hours of post-injury survival. (Haematoxylin and eosin x 160).

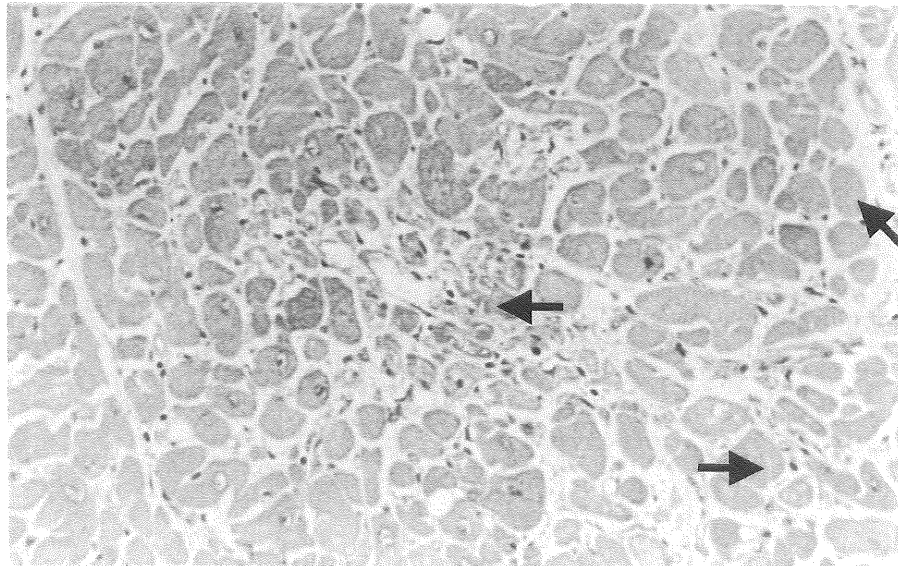


Fig. 8.5. Cellular disintegration at a slightly later stage of lesion evolution than that seen in fig 8.4. The myocytolytic process shows indistinct peripheral extension into the surrounding myocardium. An interstitial mononuclear infiltrate is present throughout the process. In addition, contraction bands (arrows) appear to be present. Case no 21; 4 days 16 hours of post injury survival (Haematoxylin and eosin x 160).

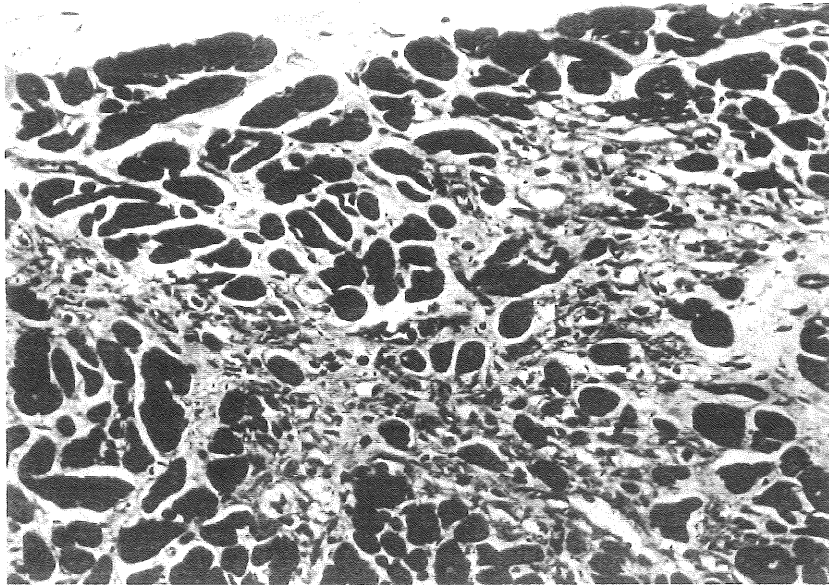


Fig. 8.6. Florid full-blown focal cardiac myocytolysis. Residual islands of myocardial fibres are present within the focus together with a prominent mononuclear infiltrate. Case no. 22; 5 days of post-injury survival (Massons's trichrome x 180)

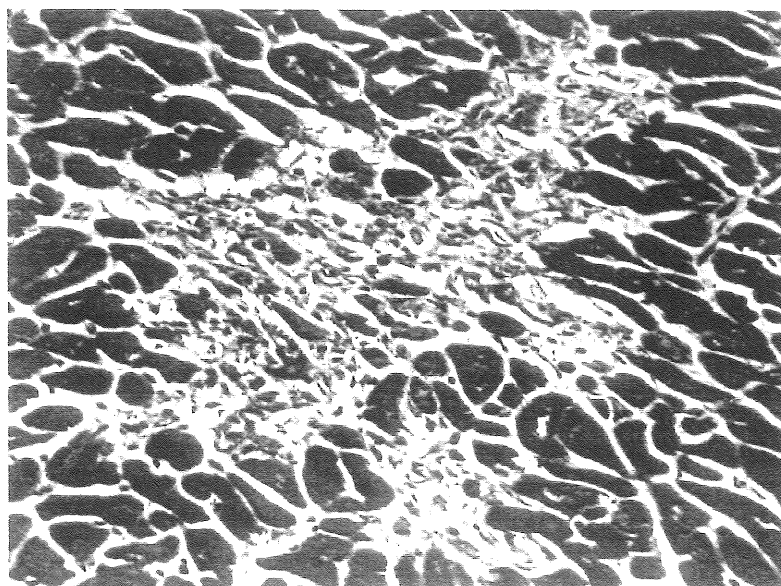


Fig. 8.7. A further focus of florid cardiac myocytolysis with peripheral extension of the process into the surrounding myocardium and with an interstitial mononuclear infiltrate. Case no. 28; 8 days 15 hours of post-injury survival (Haematoxylin eosin x 180).

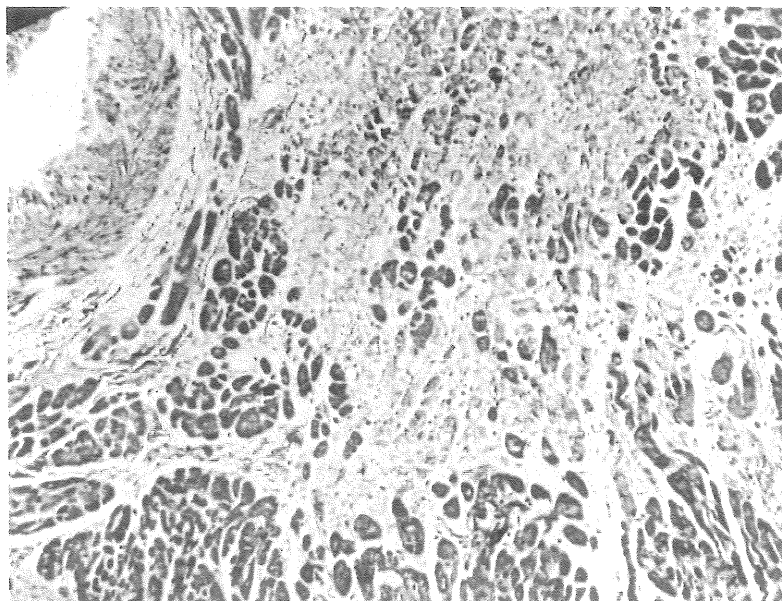


Fig. 8.8. A focus of florid cardiac myocytolysis at a later stage of evolution than that depicted in Fig. 8.7. Residual islands of myocardial fibres reduced in size are present within the focus. Case no. 30; 11 days 6 hours of post-injury survival (Masson's trichrome x 80).

The greatest number of areas of myocardium showing the florid lesion occurred in cases 22, 27, 28, and 32 in whom 12, 6, 10, and 11 areas showed this change respectively (Table 2). The greatest extent and severity of change also occurred in these areas (Table 3).

Mononuclear infiltration

Concomitant with the foregoing focal myocytolytic changes, a proliferative cellular reaction developed within the interstitial stroma between the injured myocardial cells (cases 18 and 20 to 32) (Table 2). This proliferative interstitial cellular response, in contrast to the cellular infiltration following classic myocardial infarction, comprised mononuclear phagocytes rather than polymorphonuclear neutrophils. This mononuclear response, both in time of appearance of this interstitial response, as well as in the number of areas of myocardium involved in this reaction, paralleled that of cardiac myocytolysis, a not unexpected finding in view of the role of the mononuclear-phagocyte system in cellular injury and repair. The extent and severity of this response similarly paralleled that of cardiac myocytolysis with the exception of case no. 20 which displayed an earlier non-florid lesion in which the severity of the reaction was less than that of the concomitant myocytolytic process. In all other cases, however, the mononuclear interstitial reaction appeared to be part and parcel of the myocytolytic lesion, paralleling it in time of onset, extent, and duration (cases 18 and 20 to 32; Figs. 8.4 to 8.8). As with myocytolysis, the cases showing the most severe involvement in terms of number of areas of myocardium involved as well as the extent and severity of pathological change were cases nos. 18, 22, 27, 28, and 32 (Tables 2 and 3).

Table 3

Scoring according to extent and severity of pathological change.

Case no.	Oedema		Myocytolysis		Mononuclear infiltration		Fibrosis		Nuclear change		Total score
	Extent	Severity	Extent	Severity	Extent	Severity	Extent	Severity	Extent	Severity	
1.	100	60	0	0	0	0	0	0	100	60	320
2.	100	60	0	0	0	0	0	0	100	60	320
3.	100	60	0	0	0	0	0	0	100	60	320
4.	100	55	0	0	0	0	0	0	95	43	293
5.	100	60	0	0	0	0	0	0	100	56	316
6.	100	59	0	0	0	0	0	0	88	32	279
7.	100	60	0	0	0	0	0	0	100	58	318
8.	100	60	0	0	0	0	0	0	100	60	320
9.	100	60	0	0	0	0	0	0	95	55	310
10.	100	59	0	0	0	0	0	0	100	50	309
11.	100	59	0	0	0	0	0	0	95	55	309
12.	100	58	0	0	0	0	0	0	90	46	294
13.	100	60	0	0	0	0	0	0	100	60	320
14.	100	60	0	0	0	0	0	0	100	60	320
15.	100	60	0	0	0	0	0	0	100	40	300
16.	100	60	0	0	0	0	0	0	100	60	320
17.	100	60	0	0	0	0	0	0	100	60	320
18.	100	55	8	8	8	8	0	0	100	42	329
19.	100	60	0	0	0	0	0	0	100	56	316
20.	100	60	6	13	8	11	0	0	100	57	355
21.	100	40	2	2	2	2	0	0	100	40	288
22.	100	60	14	19	14	19	0	0	100	60	386
23.	100	60	1	1	1	1	0	0	100	60	324
24.	100	60	5	5	5	5	0	0	100	60	340
25.	100	60	5	5	5	5	0	0	100	60	340
26.	100	60	5	5	5	5	0	0	100	60	340
27.	100	60	8	8	8	8	0	0	100	60	352
28.	100	60	10	12	10	12	0	0	100	60	364
29.	100	60	4	2	4	2	0	0	100	60	332
30.	100	60	4	4	4	4	0	0	100	60	336
31.	100	60	5	12	5	12	0	0	100	60	354
32.	100	60	13	17	13	17	0	0	100	60	380
33.	100	60	0	0	0	0	5	5	100	60	330
34.	100	60	0	0	0	0	5	3	100	60	328
35.	100	60	0	0	0	0	7	7	100	60	334
36.	100	60	0	0	0	0	3	3	100	60	326
37.	100	60	0	0	0	0	11	11	100	60	352
38.	18	6	0	0	0	0	13	13	100	60	210
39.	17	7	0	0	0	0	9	8	100	60	201
40.	0	0	0	0	0	0	14	27	100	60	201
41.	0	0	0	0	0	0	11	22	100	60	193
42.	0	0	0	0	0	0	6	12	0	0	18

Footnote: Total score represents a simple arithmetical summation of the individual scores obtained in each case.

Derivation of scores

Extent

Twenty areas of myocardium were examined in each case. If, for example, in Case no. 20 changes were noted within 9 - 10 high-power fields (HPF) in all 20 areas of myocardium examined then, as indicated in the scoring system applied, a score of 5+ was allocated and, by simple arithmetical multiplication, $5 \times 20 = 100$.

Severity

Similarly, if on histopathological evaluation (as judged by myself and subsequently reviewed and independently corroborated by the cardiac histopathologist) a maximum score of, say, 3 was allocated according to the judged severity and found in all 20 areas of myocardium examined then $3 \times 20 = 60$ and so on. This very simple system of arithmetical summation and multiplication was applied to and carried throughout all 5 different histopathological changes observed, namely, oedema, focal myocytolysis, mononuclear infiltration, fibrosis, and nuclear alterations.

Post-myocytolytic stromal collapse fibrosis

Following focal myocytolytic necrosis with progressive disintegration of myocardial fibres, areas of post-myocytolytic stromal collapse fibrosis appeared. These areas, enclosing residual islands of fibres varying in size were often punctuated by dilated vessels. These early foci appeared from the 13th post-injury day onwards and involved between three to five areas of myocardium (cases 33 and 34; Figs. 8.9 and 8.10) (Table 2).

Following the early stage of post-myocytolytic stromal collapse fibrosis, a more mature stage appeared on the 22nd day after injury (case no. 35; Fig. 8.11). This manifested as a more pronounced area of fibrosis in which residual islands of tissue varying in size and with dilated vessels were still in evidence. Like the earlier lesions noted at days 13 and 15 (cases 33 and 34), these more advanced foci of stromal collapse fibrosis were seen in a similar number of areas of myocardium examined (Table 2).

At 24 days after injury a more clearly defined focus of scar tissue formation appeared, possibly representing type III collagen (case no. 36). This more prominent desmoplastic nidus clearly lay further along the evolutionary path of scar tissue formation. Like the earlier foci of post-myocytolytic stromal collapse fibrosis, residual islands of myocardial tissue varying in size and blood vessels were still present within the nidus of tissue sclerosis together with scattered mononuclear cells.

From about four weeks after injury (case no. 37), well-defined discreet foci of scar tissue became evident, possibly representing type I collagen (cases 37 to 41; Fig. 8.12). In contrast to the earlier stages of fibrosis noted, these later definitive foci were more profuse and involved up to 14 of the areas of myocardium examined. However, like the earlier still evolving foci, residual islands of myocardial fibres were still present within

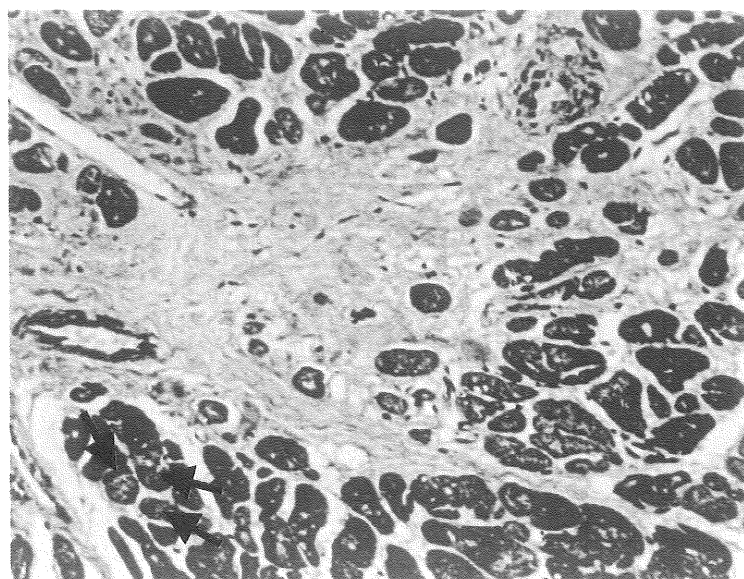


Fig. 8.9. A focus of post-myocytolytic stromal collapse fibrosis with peripheral extension of the focus into the surrounding myocardium. Residual myocardial fibres varying in size are still present within the focus together with a sparse mononuclear infiltrate. In addition, opaque contraction bands (arrows) appear to be present. Case no. 33; 13 days of post-injury survival (Haematoxylin and eosin x 180).

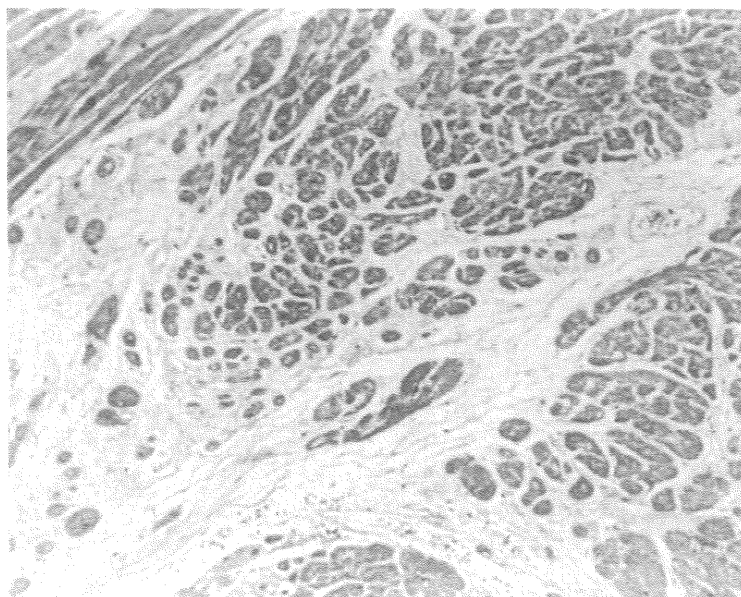


Fig. 8.10. A further focus of post-myocytolytic stromal collapse fibrosis with peripheral extension of organizing fibrous tissue into the surrounding myocardium. Residual islands of myocardial fibres varying in size are still present within the focus. A dilated congested blood vessel is noted within the focus. Case no. 34; 15 days of post-injury survival (Haematoxylin and eosin x 180).

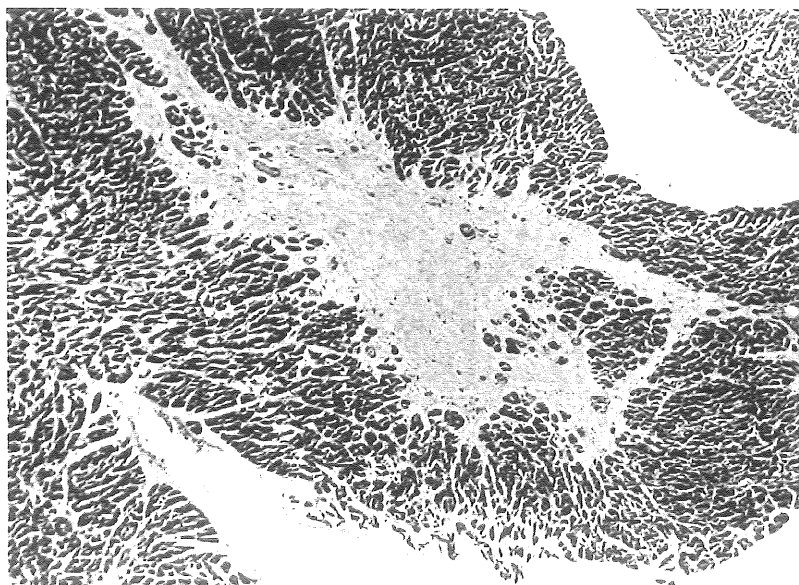


Fig. 8.11. A focus of post-myocytolytic fibrosis at a more mature stage of scar tissue formation than that seen in figs. 8.9 and 8.10. A vessel is noted within the focus together with a number of residual islands of myocardial fibres varying in size and a scanty mononuclear infiltrate. Case no. 35; 22 days of post-injury survival (Haematoxylin and eosin x 100)

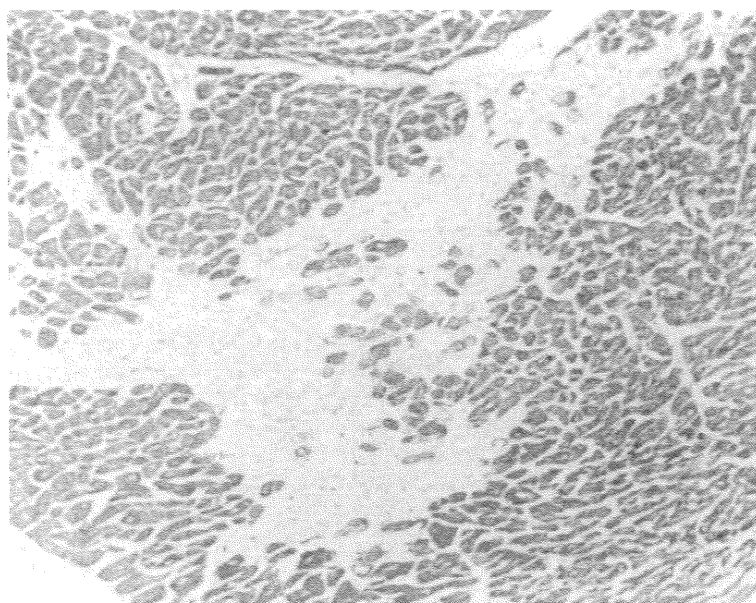


Fig. 8.12. Post-myocytolytic fibrosis at a later stage of evolution than that seen in Fig 8.11. The fibrotic process extends peripherally into the surrounding myocardium with residual islands of myocardial fibres within the lesion. Case no. 38; 8 days of post injury survival (Haematoxylin and eosin x 115).

these fibrotic foci but, in contradistinction to the earlier lesions, no vessel dilatation or presence was noted.

Morphological patterns of reactive fibrosis

As with focal myocytolysis, several distinctive patterns of reactive fibrosis began to emerge, bearing close morphological resemblance to the patterns observed with the variants of focal myocytolysis previously seen. The first pattern that emerged and seen both in the early phases of post-myocytolytic stromal collapse fibrosis and the later stages of more advanced fibrosis was that of discreet single foci widely separated and distinct from one another with each focus surrounded by otherwise uninvolved myocardium (Fig. 8.11). The second pattern, again as seen with one of the variants of focal myocytolysis, comprised foci in close proximity to one another but nevertheless separated by an intervening band of uninvolved myocardium. The third pattern that emerged was that of closely apposed foci which appeared to be in the process of coalescing or merging with each other (Figs. 8.9 and 8.12). In these cases, a peripherally extending tongue of fibrotic tissue appeared to bridge two foci.

As with the lesions of focal myocytolysis, variants of the multiple pattern of involvement appeared to consist of either large well-defined foci of approximately equal size or of a large focus with a smaller neighbouring focus in close proximity to the major lesion. Like the foci of focal myocytolysis in which special stains such as phosphotungstic-acid haematoxylin (PTAH) or Masson's trichrome were particularly effective in delineating the process (Fig. 8.8), these fibrotic foci similarly evinced a stellate or cruciate configuration with peripherally extending tongues of tissue radiating into and infiltrating the surrounding myocardium (Figs. 8.9 and 8.12).

Further examination of the foci of fibrosis showed what appeared to be a clearing and dissolution of

the residual myocardial cells within the centre of these fibrotic foci (Figs. 8.9, 8.10, 8.11, and 8.12). This myocyte clearing appeared to begin within the centre of the cell extending centrifugally to leave a peripheral doughnut-like rim of residual tissue, similar to the changes observed both in vacuolar degeneration (Fig. 8.1) and focal myocytolysis (Figs. 8.4 and 8.8). This individual cell lesion thus appeared to mirror the morphological configuration of the multicellular foci of myocytolysis and fibrosis which adopt a centrifugal pattern with peripheral extension of the process.

With temporal progression a further pattern of fibrosis became evident, manifesting from the seventh week onwards (Fig. 8.13; case no. 41). In this form of involvement, the fibrotic process adopted a more diffuse pattern with widely disseminating tongues of tissue extending peripherally into the surrounding myocardium. Despite the widespread nature of the process, residual islands of tissue were still apparent within these fibrotic foci.

In essence, therefore, foci of post-myocytolytic stromal collapse fibrosis became evident from the 13th post-injury day (case no. 33; Fig. 8.9) (Table 2). With lesion progression, evolution and development, further more advanced and definitive foci of fibrosis appeared with a more pronounced desmoplastic reaction (Figs. 8.10 to 8.12). These were found in all cases up to and including five-and-a-half months of post-injury survival (case no. 42).

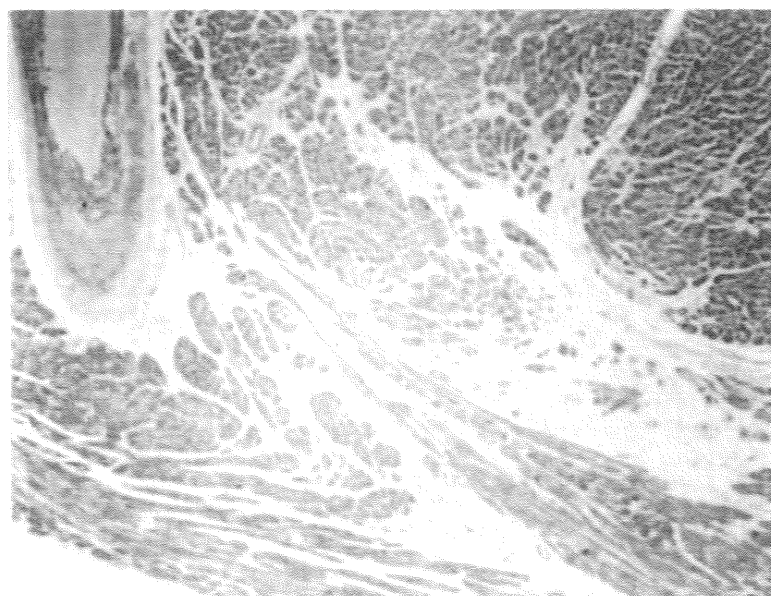


Fig. 8.13. Advanced fibrotic scarring with disseminating tongues of fibrous tissue extending peripherally into the surrounding myocardium. Residual islands of myocardial fibres are still present within the fibrotic focus. Case no. 41; 49 days of post-injury survival (Haematoxylin and eosin X 15).

Reference to Table 2 indicates an abrupt transition from those cases showing myocytolysis (cases 18 and 20 to 32) to those in which foci of stromal condensation and fibrosis began to emerge (case no. 33) and culminating in advanced fibrotic scarring (case no. 41; 49 days and 16 hours of post-injury survival). The greatest number of areas of myocardium involved by the fibrotic process occurred in cases 37, 38, 40 and 41 in whom survival periods after injury of 27, 28, 36, and 49 days were respectively recorded (Table 2). The greatest extent and severity of change in an area of fibrosis also occurred in these cases (Table 3). Reference to Tables 4a to 4e shows the frequency of involvement of each area of the heart by each type of pathological change.

The primary purpose of Tables 2 and 3 was to allocate a scoring system in an attempt to determine whether any statistically significant relationship existed between craniocerebral injury and different variables. Scoring of the histopathological variables on the basis of severity and extent of lesion thus not only accords with standard histopathology practice and methodology but provides for statistical purposes a reduction and expression of these changes in numerical terms and values. These could then be compared with the craniocerebral injury scores which, as indicated, were allocated on the basis of depth of lesion which has been shown, as previously indicated, to correlate both experimentally and clinically with severity and hence clinical outcome due to the concomitant vasogenic oedema which pathophysiologically results. Simply put, histopathological terms and craniocerebral injury lesions must be expressed numerically as there is no other way to enable statistical methodology to be applied.

Table 1 thus comprised the core data of age, sex, ethnic group, and survival period in an easily readable and tabulated form in accordance with standard methods of basic data presentation. Table 2, on the other hand, comprised a representation of the number of areas of myo-

cardium involved by each type of pathological change with the total score allocated being a simple arithmetical summation of the individual scores obtained in each case. However, so many 0 values were present (Tables 2 and 3) from a statistical viewpoint these variables could not be regarded as continuous. For statistical purposes, therefore, these variables were divided into two groups, namely 0 and >0 . With regard to Total score 1 (Table 5), and which refers to the histopathological variables, the cutoff point was taken as 40 to form two groups as the histopathological variables interstitial oedema and nuclear changes together added up to 40 throughout cases 1 to 37.

In contrast to Table 1 where the survival period is rendered in actual chronological time i.e., 20 minutes (cases 1 and 2), in Table 2 this is reduced to and expressed in statistical terms. Thus, a survival period of 20 minutes in Table 1 is, in statistical terms, expressed as 0.014 while a survival period of 8 days and 15 hours (case no. 28) is expressed as 8.625 on a simple arithmetical progression. This approach, in addition to yielding the relevant statistical analyses, enabled easy identification and recognition of the time of appearance of each lesion facilitating comparison with other studies. Similarly, in Table 2, the craniocerebral injury score derived on the basis of depth and extent of lesion (the pathophysiological basis of which is alluded to above) on an increasing scale of severity is shown. Table 3, on the other hand, shows the scoring according to the extent and severity of each type of pathological change while Table 4, expressed into one meaningful table, shows the frequency of involvement of each area of the heart by each type of pathological change.

In Table 5, the results of the various U-tests are summarized where the mean craniocerebral injury values are quoted for interpretation purposes with the corresponding p values. In this regard, while the level of significance applied in behavioural research is conducted at the

FREQUENCY OF INVOLVEMENT OF EACH AREA OF HEART

Table 4a		OEDEMA																			
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T		
38	37	38	39	37	38	39	38	37	37	38	39	38	37	37	37	37	38	37	37		
(90.5%)	(88.1%)	(90.5%)	(92.9%)	(88.1%)	(90.5%)	(97.5%)	(90.5%)	(88.1%)	(88.1%)	(90.5%)	(92.9%)	(90.5%)	(88.1%)	(88.1%)	(88.1%)	(88.1%)	(90.5%)	(88.1%)	(88.1%)		
4	5	4	3	5	4	3	4	5	5	4	3	4	5	5	5	5	4	5	5		
(9.5%)	(11.9%)	(9.5%)	(7.1%)	(11.9%)	(9.5%)	(7.1%)	(9.5%)	(11.9%)	(11.9%)	(9.5%)	(7.1%)	(9.5%)	(11.9%)	(11.9%)	(11.9%)	(11.9%)	(9.5%)	(11.9%)	(11.9%)		

Table 4b		NUCLEAR CHANGE																		
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	41	39	40	41	41	
(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(97.6%)	(92.9%)	(95.2%)	(97.6%)	(97.6%)	
1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	3	2	1	1	
(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(2.4%)	(7.1%)	(4.8%)	(2.4%)	(2.4%)	

Table 4c		FOCAL MYOCYTOLYSIS																		
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
7	3	2	4	7	8	9	6	3	1	5	10	9	4	0	2	0	1	0	0	
(16.7%)	(7.1%)	(4.8%)	(9.5%)	(16.7%)	(19.0%)	(21.4%)	(14.3%)	(7.1%)	(2.4%)	(11.9%)	(23.8%)	(21.4%)	(9.5%)	(0%)	(4.8%)	(0%)	(2.4%)	(0%)	(0%)	
35	39	40	38	35	34	33	36	39	41	37	32	33	38	42	40	42	41	42	42	
(83.3%)	(92.9%)	(95.2%)	(90.5%)	(83.3%)	(81.0%)	(78.6%)	(85.7%)	(92.9%)	(97.6%)	(88.1%)	(76.2%)	(78.6%)	(90.5%)	(100%)	(95.2%)	(100%)	(97.6%)	(100%)	(100%)	

Table 4d		MONONUCLEAR INFILTRATION																		
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
7	3	2	4	7	8	9	6	3	1	5	10	9	4	0	2	0	1	0	0	
(16.7%)	(7.1%)	(4.8%)	(9.5%)	(16.7%)	(19.0%)	(21.4%)	(14.3%)	(7.1%)	(2.4%)	(11.9%)	(23.8%)	(21.4%)	(9.5%)	(0%)	(4.8%)	(0%)	(2.4%)	(0%)	(0%)	
35	39	40	38	35	34	33	36	39	41	37	32	33	38	42	40	42	41	42	42	
(83.3%)	(92.9%)	(95.2%)	(90.5%)	(83.3%)	(81.0%)	(78.6%)	(85.7%)	(92.9%)	(97.6%)	(88.1%)	(76.2%)	(78.6%)	(90.5%)	(100%)	(95.2%)	(100%)	(97.6%)	(100%)	(100%)	

Table 4e		INTERSTITIAL FIBROSIS																		
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S	T	
5	6	8	5	7	6	4	3	2	2	5	7	6	4	4	1	0	2	0	0	
(11.9%)	(14.3%)	(19.0%)	(11.9%)	(16.7%)	(14.3%)	(9.5%)	(7.1%)	(4.8%)	(4.8%)	(11.9%)	(16.7%)	(14.3%)	(9.5%)	(9.5%)	(2.4%)	(0%)	(4.8%)	(0%)	(0%)	
37	36	34	37	35	36	38	39	40	40	37	35	36	38	38	41	42	40	42	42	
(88.1%)	(85.7%)	(81.0%)	(88.1%)	(83.3)	(85.7%)	(90.5%)	(92.9%)	(95.2%)	(95.2%)	(88.1%)	(83.3)	(85.7%)	(90.5%)	(90.5%)	(97.6%)	(100%)	(95.2%)	(100%)	(100%)	

Footnote: Space limitations preclude an indication of which specific areas of myocardium are represented by A to T. However, this is specifically detailed in the text.

Space limitations preclude numbering of areas.

n = 42 (Number of areas of myocardium examined, in which a positive change was noted.)

**SUMMARY OF THE RESULTS OF THE VARIOUS U-TESTS
WHERE THE MAIN CRANIOCEREBRAL INJURY VALUES ARE
QUOTED FOR INTERPRETATION PURPOSES WITH THE
CORRESPONDING P VALUES.**

Table 5

Classifying variables	Class	N	Mean CC injury	p-value
Sex	Male	35	5.29	0.3279
	Female	7	4.00	
Ethnic group	Black	30	5.43	0.2365
	White	11	4.09	
Cardiac myocytolysis	0	28	5.36	0.5596
	>0	14	4.50	
Mononuclear infiltration	0	28	5.36	0.5596
	>0	14	4.50	
Fibrosis	0	32	5.41	0.1913
	>0	10	4.00	
Total score 1	≤40	25	5.44	0.4980
	>40	17	4.53	

NOTE: FIBROSIS IS < 0.2

.01 and .05 levels of significance, in exploratory research as in this study a level of .20 may be deemed more appropriate,⁴ this being the level applied by the statistical analyst.

While no significant difference and hence relationship was found to exist between craniocerebral injury and the variables age, gender, ethnic group, focal myocytolysis and mononuclear infiltration and nor could any predictive values be ascribed to any of these variables with regard to outcome, a statistically significant relationship was found to exist between craniocerebral injury and myocardial fibrosis, significance being taken as indicated above at the $p < 0.2$ level appropriate to exploratory research.

Other, therefore, than myocardial fibrosis no myocardial change could be related to a scored craniocerebral injury that might be a predictor for cardiac integrity and integrity of the transplanted heart from previously head injured victims. In other words, there was no specific craniocerebral injury score at or beyond which a predictable significant change would occur in the myocardium apart from myocardial fibrosis. Thus, it was not possible on the data to provide a specific correlate that would reflect a clinical outcome although, on pathological grounds, a natural progression is seen to occur through cardiomyocyte dropout to necrosis and healing by scar tissue formation and fibrosis, the latter finding carrying implications for ventricular remodeling and therefore myocardial integrity.

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CHAPTER 9DISCUSSION: CARDIAC TRANSPLANTATION AND CATECHOLAMINE
CARDIOTOXICITY:--THE POTENTIAL LONG-TERM CARDIAC IMPLI-
CATIONS OF ANTECEDENT CRANIOCEREBRAL INJURY-----

While cardiac transplantation is established as treatment for end-stage cardiac failure,¹ the selection of donor hearts is beset with a number of difficulties. Exclusion criteria to which selection of a donor heart may be subject include age and unfavourable electrocardiographic changes, these being major causes for refusal of potential donors.² Furthermore, it has been shown, both experimentally^{3,4} and clinically,^{5,6} that brain death, a major prerequisite for cardiac explantation, is associated with severe haemodynamic deterioration and that underlying this haemodynamic derangement is a profound alteration in cardiac contractility manifested pathologically by contraction band necrosis.⁷ However, it has also been shown that this haemodynamic derangement can be fully reversed despite the impairment of cardiac contractile function if the heart is excised and perfused ex vivo.

While these latter changes may be accompanied by a switch from aerobic to anaerobic metabolism,⁸ and were formerly thought to constitute a contraindication to transplantation, further studies have indicated that brain-death induced impairment of cardiac contractile performance may not preclude the use of hearts for transplantation.⁹ The authors of this latter study, while conceding that it was undertaken in the rat with an ex vivo evaluation of functional recovery, nevertheless felt the ability to reanimate hearts before or after extended periods of hypothermic storage was in no way compromised. Acute changes are, therefore, both treatable and reversible, an encouraging finding in the

light of current clinical practice of storage of donor hearts for periods of up to 4 to 6 hours with preservation by single dose cardioplegic infusion. Thus, the need to exclude hearts for transplantation simply on the grounds of impaired haemodynamic characteristics may no longer constitute a valid criterion for exclusion.

Catecholamine-mediated effects of increased intracranial pressure on myocardial structure and function

What of the catecholamine-mediated effects of increased intracranial pressure following head injury and, by implication, in brain-dead donors on myocardial structure and function?¹⁰⁻¹² Studies in potential clinical donors and in experimental animals have suggested that brain death can have major functional and histopathological effects on the myocardium.¹³⁻¹⁷ Histopathological studies of the failed hearts very often reveal focal damage of the myocardium. These cardiac lesions, often typical, consist of petechial haemorrhages in the sub-endocardium, contraction bands,¹⁸ and focal myocytolysis. These changes are also found in patients dying from acute cerebral lesions,^{19,20} in experimental situations involving catecholamine administration,²¹ or when brain death is induced by acutely increasing the intracranial pressure.^{22,23} These latter changes, believed to be caused by a catecholamine excess that occurs during the process of brain death, are thought to be related to a cytosolic calcium overload.²⁴ Thus, one of the consequences of traumatic head injury is the initiation of a cascade of deleterious events related to activation of the sympathetic nervous system.²⁵⁻²⁸

Animal models of increased intracranial pressure

In animal models of increased intracranial pressure, cerebral injury results in marked perturbations of haemodynamics and histopathological evidence of myocardial damage.²⁹⁻³⁸ Studies have also demonstrated alterations in beta-adrenergic signal transduction in model systems exposed to high levels of catecholamines. In cultured heart cells, short-term (1 to 4 hours) exposure to catecholamines generally causes uncoupling followed by downregulation of beta-adrenergic receptors as well as calcium overload in isolated cardiomyocytes.³⁹⁻⁴³ Similarly, in in vivo models, chronic elevation of norepinephrine also causes uncoupling of myocardial beta-adrenergic receptors from mechanical response and adenylyl cyclase stimulation.⁴⁴

Experimental models of the effect of brain death in producing catecholamine surge

Experimental models of the effect of brain death have shown that brain death can lead to a release of catecholamines by distortion and compression of brain mass, vascular compression, and/or architectural distortion. Low levels of injury allow for a transient increase in ICP and other haemodynamic variables which then reverse as compensatory processes or autoregulation become effective. As injury severity increases autoregulation becomes progressively impaired. It has been shown that when the sympathetic storm is built up slowly, the haemodynamic changes are not so acute and intense.⁴⁵ The sympathoadrenergic system thus appears to have a graded ability to respond to stress.⁴⁶

Further studies using experimental models of brain death have similarly reported tremendous in-

creases in catecholamine levels with typical cardiac and pulmonary lesions.⁴⁷⁻⁴⁹ All of these studies, however, used acute increases in the intracranial pressure (ICP) to induce brain death. Evidence from clinical situations shows that patients dying of acute intracranial lesions large enough to produce acute increases in ICP show transmural scattered foci of myocardial injury,⁵⁰ and which are not seen in patients with slowly progressive intracranial tumours. From autopsy studies of patients dying at various periods after sudden increases in ICP, it appears that focal myocardial damage requires at least 6 hours to develop. and 8 to 12 per cent of the patients dying from acute cerebral lesions show such cardiac damage.⁵¹

While in humans the increase in circulating catecholamine levels reflects the severity of the neurological insult and is a physiological marker in assessing patient outcome in traumatic brain injury,⁵² little is known about the relationship between the extent of brain injury, catecholamine levels, and the severity of myocardial damage. Hamill et al⁵³ reported that in 33 patients with traumatic brain injury, the levels of circulating catecholamines appeared to be excellent endogenous and readily quantifiable markers that reflected the extent of brain injury and predicted the likelihood of patient recovery. The cardiac implications, however, were not investigated. It is unclear whether the sympathetic response reflects a diffuse axonal injury therefore or specific lesions (brain stem and/or other, although, as indicated in chapter six, the area that appears to be most intimately linked with the cardiovascular system is the orbital surface of the frontal lobe (area 13), followed by the anterior cingulate gyrus (area 24).

Changes in the beta-receptor-G-protein-adenylyl cyclase complex in donor heart dysfunction (DHD) ---

What of changes occurring in the beta-receptor-G-protein-adenylyl cyclase complex in explanted human hearts exhibiting depressed left ventricular systolic function from donor heart dysfunction (DHD)?⁵⁴ These have been investigated by White et al⁵⁵ who have found that the prominent uncoupling of beta-adrenergic receptors in DHD hearts is most likely caused by an alteration in G-protein function, perhaps on the basis of an increase in the functional activity of G_i ,⁵⁶ without a change in the catalytic unit of adenylyl cyclase. The magnitude of impairment in adenylyl cyclase activity in response to pharmacological probing noted in the White study is in agreement with the findings previously described in the chronic norepinephrine infusion model and is quite similar to the changes reported in end-stage chronic cardiac failure.⁵⁷

The contractile response findings in the White study were consistent with desensitization to beta-agonist stimulation plus myocardial damage produced by catecholamine toxicity. Several experimental studies have shown a depression of myocardial mechanical response by endogenous or exogenous elevation of systemic or regional adrenergic activity.⁵⁸ Similarly, massive sympathetic discharge induced by brain damage in anaesthetized rabbits results in typical catecholamine-induced myofibrillar damage and causes a significant decrease in left ventricular performance assessed two-and-a-half hours after the initial insult.⁵⁹ The decrease in maximum contractile response to calcium noted in the White study is also in agreement with another investigation showing that 14 days of continuous norepinephrine infusion results

in an 18 per cent decrease in maximal inotropic response to calcium in isolated rabbit left ventricular papillary muscles.⁶⁰ Thus, unlike in chronic human heart failure,⁶¹ DHD ventricles are characterized by a decrease in the contractile response to both beta-agonists and calcium i.e., 'downregulation'. This suggests that the contractile apparatus is relatively more damaged via catecholamine-mediated myocardial injury in DHD than in chronic cardiac failure,⁶² based on the normal maximal systolic tension response to calcium in the latter and with the former mediated via altered left ventricular myocardial gene expression.⁶³

Comparative findings of the White,⁵⁵ Shivalkar,³³ and Galiñanes and Hearse³ studies

The findings of the White study are also in agreement with those of Shivalkar et al, as the former also report features typical of catecholamine toxicity in histological analysis of two DHD hearts. The White data complement those of Shivalkar et al by demonstrating that profound beta-adrenergic receptor desensitization may contribute to DHD and to the poor functional recovery of these hearts when transplanted. However, an important observation of the White study was that despite these changes, creatine kinase levels in DHD ventricles did not differ from nonfailing organ donor controls, indicating that abnormal contractile function may be reversible, as shown by Galiñanes and Hearse.

Clinical implications of the White study

The results of the White study may have important

clinical implications. The average age of the DHD hearts in this latter study was 22 years; younger hearts exhibit an increased sensitivity to beta-agonist stimulation in vivo as well as higher beta-adrenergic receptor density than older hearts.⁶⁴ In this regard, they may therefore be more vulnerable to a sudden increase in sympathetic activity. This is of relevance to the findings in the study in that the greatest number of subjects fell into the 21 to 30 year age group, paralleling the distribution seen in studies of traumatic injury to other organ systems indicated in the previous chapter. The significance of this finding lies not only in the fact that this young age group is the one most frequently involved in traumatic injury but will then also comprise the age group from which organs are most frequently harvested for transplantation.

Elevation of circulating catecholamine levels in non-traumatic pathological states

Elevation of circulating catecholamines also occurs in some nontraumatic pathological states such as congestive cardiac failure and phaeochromocytoma. In congestive cardiac failure, this might represent a complex dysfunction of sympathetic neural regulation rather than merely a reflex compensatory response.⁶⁵ Marked patient-to-patient variation occurs, however, as while arterial norepinephrine levels are within normal limits in one-third of patients with severe congestive cardiac failure, they are markedly elevated in others.⁶⁶ A significant feature, however, is that the extent of plasma norepinephrine elevation correlates directly with the severity of left ventricular dysfunction and predicts cardiac mortality.⁶⁷ Moreover, in patients harbouring a functional phaeochromocytoma, the catecholamine cardiomyopathy manifests itself as active focal

myocarditis and/or increased myocardial fibrosis,⁶⁸ of particular relevance to the findings in this study where areas of focal myocytolysis eventuated in myocardial fibrosis. In phaeochromocytoma patients, furthermore, cardiovascular disorders are often the predominant cause of death.

Beta-blockade has been beneficial in reducing cardiac damage in the setting of brain injury,⁶⁹ or in nontraumatic conditions characterized by a sudden increase in sympathetic activity such as myocardial infarction.^{70,71} Since high doses of dopamine cause nor-epinephrine release,^{72,73} and may therefore increase cardiac adrenergic drive, dopamine and other catecholamines may not be the ideal agents for stabilization of blood pressure in organ donors.⁷⁴⁻⁷⁶

Haemodynamic stress-induced cardiac adaptive and maladaptive responses and the role of paracrine, autocrine, and neurohormonal mediators in cardiac hypertrophy, left ventricular dysfunction and ventricular remodeling

Cellular and molecular changes have been identified in dysfunctional myocardium which have important implications for cardiac adaptation and maladaptation.⁷⁷⁻⁸² Functional abnormalities in excitation-contraction coupling, contractile protein function, and energetics have been identified,⁸³⁻⁸⁸ while at the molecular level alterations have been identified in the expression of several proteins that are central to normal myocardial structure and function.⁸⁹⁻⁹¹ There is strong evidence from in vitro, animal, and human studies to suggest that these molecular and cellular events are secondary to both mechanical forces and a variety of neuronal, endocrine, and autocrine/paracrine mediators that act on the myocardium.⁹²⁻⁹⁸ Some of the responses identified are adaptive and

sustain cardiac function while others are maladaptive. Equally, some may play contributory roles for impaired myocardial function, while others may simply accompany the dysfunctional state as epiphenomena.⁹⁹

Mediators involved in cardiovascular adaptation under conditions of haemodynamic stress and myocardial dysfunction

A variety of mediators are involved in adaptation of the cardiovascular system under conditions of haemodynamic stress and myocardial dysfunction. Some act on neighbouring cells of another type (paracrine effect) or on the cell of origin (autocrine effect),¹⁰⁰ while others are circulating hormones (endocrine effect). Mediators involved include peptides that act primarily locally in the vicinity of their production, such as endothelin, peptide growth factors (i.e., transforming growth factor- α), and inflammatory cytokines (i.e., interleukin-1 β and tumour necrosis factor- α). These and other local mediators act in concert with the autonomic nervous system and circulating hormones to modulate cardiovascular organ function. Most importantly, many of these mediators have effects on the growth of cardiovascular tissues with implications for structural remodeling, cardiac hypertrophy, cardiomyocyte necrosis, and cardiac adaptation and maladaptation.¹⁰¹⁻¹⁰³

Cytokines

Several peptide mediators, including peptide growth factors and inflammatory cytokines,¹⁰⁴ have important effects on the myocardium and its vasculature. As their name indicates, peptide growth factors are regulators

of myocardial growth modulating gene expression in cardiomyocytes, vascular smooth muscle cells, endothelial cells, and fibroblasts.¹⁰⁵ Peptide growth factors can be expressed by several cell types in the heart, including cardiomyocytes¹⁰⁶ and fibroblasts.¹⁰⁷ Of relevance to the finding of cardiac hypertrophy in this study, several peptide growth factors have been shown to cause hypertrophy, with increased expression of these factors in response to haemodynamic stress and norepinephrine.

Of further relevance to myocardial hypertrophy is that the inflammatory cytokine interleukin-1 beta, has been shown to induce myocyte hypertrophy in vitro.^{108,109} Furthermore, cytokines may exert long-term effects on the remodeling of myocardial tissue (Fig. 9.1).

Endothelin

The most potent peptide vasoconstrictor released by endothelial cells throughout the circulation is endothelin.^{110,111} Of interest is that the release of endothelin from endothelial cells in vitro can be enhanced by several vasoactive agents (i.e., norepinephrine, angiotensin II, thrombin) and cytokines (i.e., interleukin-1 beta). The growth promoting effects of interleukin-1 beta have been alluded to above while those of angiotensin II and norepinephrine will be discussed below.

It has furthermore been shown that in cultured cardiomyocytes, endothelin itself induces cellular hypertrophy associated with the induction of foetal genes,¹¹² while in rats with pressure-overload induced hypertrophy caused by aortic banding, inhibition of myocyte hypertrophy and prevention of foetal gene expression has been transiently produced

Central role of cytokines in modulating the changes in the structure and function of the myocardium

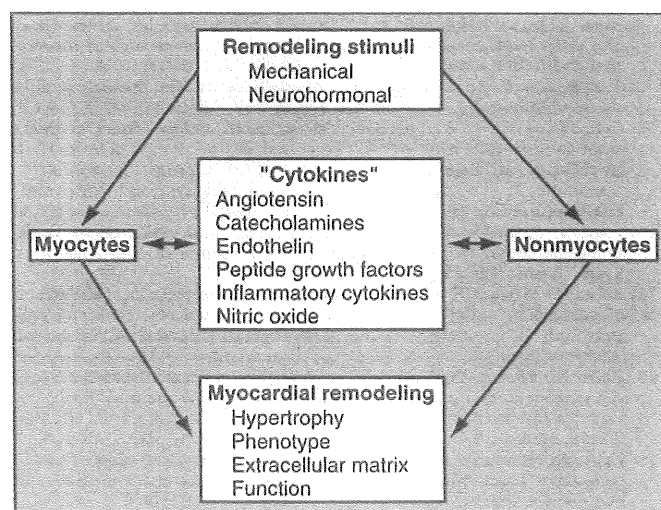


Fig. 9.1. Central role of cell-to-cell interaction in myocardial remodeling. Both myocytes and nonmyocytes (i.e., fibroblasts, endothelial cells, vascular smooth muscle cells) in the myocardium produce a number of intercellular mediators, many of which are cytokines, that may act on neighbouring cells in an autocrine or paracrine manner thereby playing a central role in modulating changes in the structure and function of the myocardium. (Thaik CM, Calderone A, Takahashi N, et al. Interleukin-I modulates the growth and phenotype of neonatal rat cardiac myocytes. *J Clin Invest* 1995; 96: 2555 – 2564.)¹⁰⁹

by administration of the endothelin_A-receptor antagonist BQ123.¹¹³ In addition, it has been shown experimentally in dogs that potent coronary artery vasoconstriction is mediated by endothelin receptors.¹¹⁴

These foregoing observations may, therefore, provide a basis both for the myocardial nuclear changes of hypertrophy noted in this study (in conjunction with the growth-promoting effects of catecholamines) as well as providing a possible explanation for coronary vasoconstriction and its attendant myocardial necrosis.

The role of angiotensin II in cardiac hypertrophy

Evidence indicates that the G_q-coupled receptor agonist angiotensin II has cardiotropic effects which may transduce experimental and human pathologic myocardial hypertrophy. Angiotensin II has both direct and indirect actions on the myocardium, modulating both cardiac contractility and hypertrophy.¹¹⁵⁻¹²¹ Angiotensin II binding sites have been described in the heart, and the development of nonpeptide angiotensin II antagonists has led to the characterization of at least two types of angiotensin II receptors, designated AT₁ and AT₂.^{122,123} The AT₂ receptor does not appear to be G-protein linked, and its true biological function remains relatively unclear although there are suggestions that it may be associated with a negative inotropic effect.

Clinical and experimental studies have clearly indicated that angiotensin receptor antagonists can produce regression of cardiac hypertrophy, an effect that in some experimental models seems independent from their effect on blood pressure.¹²⁴⁻¹²⁷ Renin and angiotensinogen mRNAs have been demonstrated in the four cardiac chambers, and AT₁ mRNA

is induced more than threefold in left ventricular myocardium from hypertrophied hearts.¹²⁸

Angiotensin II can diffuse through the cardiac interstitium to activate receptors on cardiomyocytes, leading to increased contractility and/or growth, increased protein synthesis in chick cardiomyocytes, and producing hypertrophy of rat cardiomyocytes with hyperplasia of cardiac non-myocytes.¹²⁹ Both these actions are mediated by the AT_1 receptor. Furthermore, an in vitro model of stretch-induced cardiac hypertrophy has demonstrated that mechanical stretch causes release of angiotensin II from cardiomyocytes and may act as an initiator of the stretch-induced hypertrophic response.¹³⁰

Further evidence for a cardiotropic effect of angiotensin II includes the observation of attenuation of development of left ventricular hypertrophy in pressure-overloaded mice by blockade of AT_1 receptors,¹³¹ with similar regression of hypertrophy in spontaneously hypertensive rats,¹³² and stimulation of new protein synthesis in isolated perfused rat hearts by direct administration of angiotensin II.¹³³

Electron microscopy shows that immunoreactive angiotensin II is preferentially localized in what appear to be secretory granules in ventricular cardiomyocytes. Thus, these observations and multiple avenues of investigation are consistent with the hypothesis that locally produced angiotensin II acts as an endogenous growth factor regulating myocardial growth, as a transducer of in vivo pathologic cardiac hypertrophy and, at the same time, affecting the level of expression of other growth factor genes.

The role of beta-adrenergic stimulation of cardiac
non-myocytes in cardiomyocyte hypertrophy-----

As previously indicated, normal myocardium consists of two main cellular components:- the myocyte, (contractile compartment) and the surrounding non-myocyte, support compartment. This latter compartment consists of endothelial cells, smooth muscle cells, and the predominant non-myocyte cell type, the fibroblast/mesenchymal cell.

Apart from the obvious functional differences between these two compartments, they also differ markedly in their proliferative capacity. Unlike the non-myocytes that maintain the ability to divide throughout life, myocytes lose the ability to undergo cell division soon after birth. As such, further growth of the myocyte compartment occurs through enlargement, or hypertrophy, of the individual cells which may be physiologic, resulting from normal growth and exercise, or pathologic associated with ischaemia/infarction. Such pathologic hypertrophy differs from physiologic growth in that it is associated with myocardial dysfunction and is an independent risk factor for both cardiovascular morbidity and mortality.¹³⁴

Utilizing a model of neonatal rat cardiomyocytes in culture, Simpson et al have reported that cardiomyocytes hypertrophy in response to adrenergic receptor stimulation.^{135,136} Furthermore, this adrenergic stimulation leads to the re-expression of foetal/neonatal contractile protein isogenes and certain cellular oncogenes similar to that seen with pressure-overload hypertrophy in vivo,¹³⁷ suggesting a common mechanism/s for the development of myocardial hypertrophy

Long et al have identified the cardiac non-myocytes as the source of a heparin-binding growth factor (NMDGF).¹³⁸ Accordingly, these workers were

interested in determining whether adrenergic stimulation of these non-myocytes could result in an enhanced paracrine myocyte growth response. The possibility that the beta-adrenergic system might have an effect on the non-myocyte fraction of the heart had previously been suggested by other workers who observed that adrenergic stimulation of cardiomyocyte gene transcription appeared to differ with respect to receptor subtype purely as a function of plated cell density.¹³⁹ Briefly, when plated at low density, the myocyte growth effect and induction of contractile protein isoform switching is predominantly α_1 in origin. In apparent contrast, when cultured at higher densities, both alpha and beta-adrenergic stimulation seem to result in these same changes in myocyte growth and transcriptional program. This apparent discrepancy might in fact relate to an indirect beta-adrenergic response. On investigation, these latter authors found that stimulation of non-myocyte adrenergic receptors results in the production of a growth-promoting conditioned medium whose potency exceeded that of medium conditioned by the non-myocytes in the absence of adrenergic stimulation.¹⁴⁰ Furthermore, work with the α_1 agonist phenylephrine indicated that this response was specific to the beta-adrenergic receptor. Additional characterization of this response indicated that it was not due to an increase in NMDGF production but might rather involve the TGF(transforming growth factor)beta family of growth factors. Additionally, in experiments aimed at defining an autocrine role of adrenergic stimulation on the non-myocytes, Long et al found that beta stimulation of these non-myocytes also resulted in an increase in non-myocyte DNA synthesis. These observations suggest that adrenergic stimulation of the non-myocyte compartment of the heart may result in both an increase in non-myocyte cell numbers and at least some of the growth factor products they elaborate. However, the

major thrust and finding of the Long et al study was the observation that in addition to playing an active role in the process of cardiac myocyte growth under 'control' conditions, the non-myocyte fraction of the heart is capable of augmenting the myocyte hypertrophic response to adrenergic stimulation via a paracrine mechanism/s.

Long et al found, in addition, that isoproterenol stimulated an increase in non-myocyte DNA synthesis as an autocrine effect. The impact of this latter observation should not be underestimated given the fact that the number of non-myocytes exceeds that of myocytes in the normal heart,¹⁴¹ and further, that the non-myocytes have been shown to increase their numbers under pathologic conditions such as pressure-overload hypertrophy and post-injury/infarction which have been associated with a hyperadrenergic state.¹⁴² Since these cells are responsible for the elaboration of much of the extracellular matrix,¹⁴³⁻¹⁴⁶ changes in their numbers and/or expression patterns in response to adrenergic agents could have profound consequences on both the systolic and diastolic properties of the heart.

Cardiac hypertrophy as a G_q -mediated autocrine/paracrine response and G_q signaling in haemodynamic stress

The most detailed mechanistic investigations of cardiomyocyte signal transduction pathways underlying hypertrophy have been performed using cultures of spontaneously beating neonatal rat ventricular myocytes. Using variations of this tissue culture model, hypertrophic effects of phenylephrine, angiotensin II, endothelin, and prostaglandin $F_2\alpha$ have all been demonstrated.¹⁴⁷⁻¹⁴⁹ A critically important feature of these structurally diverse hypertrophic

factors is that, like angiotensin II, they each stimulate a seven transmembrane-spanning receptor that activates phospholipase C via the G_q class of GTP (guanosine triphosphate)-binding proteins. Hypertrophic effects of G_q -coupled receptor agonists are associated with activation of phospholipase C,^{150,151} with expression of activated G_q being sufficient to stimulate at least some features of hypertrophy as measured by increased cell size.¹⁵² Furthermore, not only may these G_q -coupled receptor agonists be involved in complex adaptive mechanisms but that in pathophysiological states of haemodynamic stress and injury, vagal 'braking' may be insufficient to prevent or preclude maladaptive responses resulting in left ventricular dysfunction.

Of particular interest regarding the potential relevance of G_q -coupled receptor agonists in pathologic cardiomyocyte hypertrophy is, as previously indicated, the observation that angiotensin II and endothelin-1 can be released from mechanically deformed cardiomyocytes.¹⁵³ Accordingly, secretion of these G_q -stimulating agonists from the heart could constitute an autocrine mechanism regulating hypertrophic responses.

That mechanical deformation could also stimulate autocrine production of G_q -coupled receptor agonists in the intact heart has been shown in a study utilizing isolated perfused guinea pig hearts.¹⁵⁴ Taken in conjunction with the aforementioned in vitro studies of mechanically deformed cultured neonatal cardiomyocytes, this latter ex vivo study of isolated perfused hearts suggests that the heart responds to mechanical deformation with production of G_q -activating agonists. These observations lend further support to a causal relationship between secretion of these autocrine/paracrine factors and the development of pathologic cardiac hypertrophy (Fig. 9.2.).¹⁵⁵

With regard to the time onset of hypertrophy

The proposed relationship between primary injury, haemodynamic stress and sympathetic activation

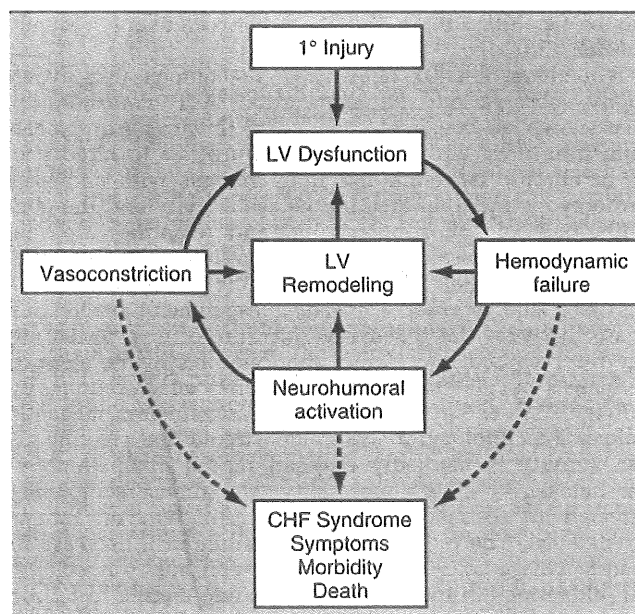


Fig. 9.2. A schematic representation of the proposed relationship between primary injury, haemodynamic stress and sympathetic neurohumoral activation. According to this thesis, haemodynamic and neurohumoral consequences of the initial injury result in both left ventricular dysfunction and ventricular remodeling. (Adams JW, Sakata Y, Davis MG, et al. Enhanced $G_{\alpha_{\text{I}q}}$ signaling: a common pathway mediates cardiac hypertrophy and heart failure. Proc Natl Acad Sci 1998b; 95: 10140 – 10145.)¹⁵²

as an adaptive/maladaptive response following injury and myocardial dysfunction, this ranges over days to months and can be divided into three stages:

Stage I: early hypertrophy (days);

Stage II: established hypertrophy (weeks);

Stage III: further hypertrophy with myocardial cell death and progressive fibrosis (months).¹⁵⁶

In addition to adrenergic nervous system stimulation and modulation of cardiomyocyte as well as non-myocyte growth,¹⁵⁷ therefore, catecholamine agents induce not only myocardial necrosis but also progressive ventricular remodeling in response to such necrosis.¹⁵⁸ Thus, as with angiotensin II which stimulates collagen-gel contraction by cardiac fibroblasts (as well as modulating myocardial hypertrophy), a further pathogenetic mechanism for induction of myocardial fibrosis and scar tissue formation following injury should be considered.

G_q-mediated cardiomyocyte apoptosis and progression to hypertrophy decompensation

An important component of the transition to hypertrophy decompensation and heart failure is myocyte necrosis.¹⁵⁹ Regardless of the cause of myocyte death, the load on the residual cells rises leading to reactive hypertrophy, which may further impair the function of these myocytes. With cellular death comes drop-out of individual myocytes which has been observed in both the senescent rat and human heart,¹⁶⁰ and which may contribute to the development of myocardial dysfunction.

A mechanism of cellular deletion which has been implicated in the physiological atrophy of various tissues and organs but which can also be initiated by pathological stimuli is apoptosis.¹⁶¹⁻¹⁷⁰ The structural changes which occur in apoptosis,

while occurring in two discreet stages, nevertheless show striking morphological similarities to the spectrum of changes observed in the early stages of the catecholamine-induced lesion. These include: i) the aggregation and clumping of nuclear chromatin into dense masses and their peripheral condensation and margination beneath the nuclear envelope; ii) the presence of lucent cytoplasmic vacuoles; iii) the tendency for the process to affect cellular clusters while in the initial stages scattered single cells are affected, and; iv) the occurrence of the process following the administration of exogenous noxious agents, as may also be observed with the lesions of focal myocytolysis.

As indicated, increased $G_{\alpha_{\text{haq}}}$ expression and signaling are not only associated with ventricular remodeling and hypertrophy decompensation in humans but also in $G_{\alpha_{\text{haq}}}$ transgenic mice and other experimental animals which express high levels of $G_{\alpha_{\text{haq}}}$ in the heart.¹⁷¹ Furthermore, increased $G_{\alpha_{\text{haq}}}$ expression and phospholipase signaling have been described in the scar and peri-infarcted myocardium of rat hearts eight weeks after coronary artery ligation.¹⁷² Hence, transgenic mechanisms which increase expression of and signaling through myocardial $G_{\alpha_{\text{haq}}}$ are associated with cardiomyocyte hypertrophy and ventricular failure in both humans and experimental animals.

A pathophysiological explanation for the progression of $G_{\alpha_{\text{haq}}}$ -mediated myocardial hypertrophy to cardiac failure has been suggested by the observation that greatly enhanced $G_{\alpha_{\text{haq}}}$ signaling in vitro can cause cardiomyocyte apoptosis. This is consistent with the findings of Althoefer et al in noncardiac cells transfected with constitutively activated $G_{\alpha_{\text{haq}}}$ in which high levels of $G_{\alpha_{\text{haq}}}$ signaling can stimulate apoptosis in cultured cardiomyocytes.¹⁷³ Studies using this in vitro

model, therefore, have not only shown that exaggeration of signals leading to hypertrophy would induce apoptosis, but also that when PGF(prostaglandin F)_{2alpha} or other agonists of G_q-coupled receptors is added to G_{alpha} expressing cells, phospholipase C activation is enhanced and the cells undergo apoptotic cell death.

An in vivo correlate of G_q-mediated cardiomyocyte apoptosis is the unique peripartum cardiomyopathy which develops in G_{alphaq} transgenic mice. G_{alphaq} overexpressing mice in the terminal period of pregnancy or immediately following delivery develop an extremely rapid and aggressive cardiomyopathy which is lethal in approximately 50 per cent of overexpressors. The hearts from animals with this peripartum cardiomyopathy exhibit massive biventricular and biatrial dilatation. Histopathologically, the hearts demonstrate evidence of cardiomyocyte dropout and replacement. Biochemical studies of ventricular tissue from the apoptotic cardiomyopathic peripartum overexpressors show exaggerated protein kinase C activity and, consistent with in vitro studies in apoptotic cardiomyocytes expressing activated G_{alphaq}, selective activation of Jun kinase and p38. Significantly, and as has been indicated, protein kinase C activation has also been implicated in the G_{alphaq}-mediated fibroblast apoptosis reported by Althoefer et al.¹⁷³ Thus, in vivo studies indicate a role for G_{alphaq} signaling not only in stimulating cardiac hypertrophy, but also in decompensation of the hypertrophied heart through stimulation of cardiomyocyte apoptosis.

The role of catecholamines and alpha-adrenoceptors in coronary artery spasm, contraction band necrosis and cardiomyocyte necrosis

The coronary arteries taking origin from the ascending aorta are richly innervated by adrenergic and parasymp-

pathetic nerves,¹⁷⁴ with both α_1 - and α_2 -adrenoceptors present.¹⁷⁵ When activated by neuronally released or circulating norepinephrine both cause vasoconstriction,^{176,177} which appears to be mediated by an increased concentration of calcium in coronary vascular smooth muscle.^{178,179} Further evidence indicates potent vasoconstriction being mediated by norepinephrine-driven endothelin receptors.¹⁸⁰

Intravenous use of norepinephrine induces a brief fall, followed by a sustained rise, in coronary vascular resistance, accompanied by a decline in coronary sinus oxygen saturation,¹⁸¹ while intravenous infusion of methoxamine, a direct-acting sympathomimetic α_1 -receptor agonist, reduces the diameter of coronary arteries, despite increasing intraluminal pressure.¹⁸²

Alpha-adrenoceptor stimulation can induce coronary spasm that can be prevented by phenoxybenzamine, an alpha-adrenoceptor antagonist that covalently binds to the alpha-receptor site resulting in irreversible blockade, as well as prazosin, an extremely potent alpha-receptor antagonist, highly selective for α_1 -receptors but with relatively low affinity for α_2 -receptors.¹⁸³ In the clinical context, the finding in some patients with chronic stable angina that administration of alpha-receptor blockers can reduce exercise-induced ST-segment depression and angina indicates that alpha-receptor-mediated coronary vasoconstriction may play a contributory role in the development of myocardial ischaemia resulting in these patients.^{184,185} It has also been reported that cocaine, a local anaesthetic that has assumed prominence as a substance of abuse, is a potent coronary vasoconstrictor both in dogs, rats, and humans.¹⁸⁶⁻¹⁸⁸ Because this effect can be prevented by the alpha-receptor blocker phentolamine, it appears to be mediated, at least in part, by alpha-adrenergic stimulation.¹⁸⁹

In this regard, it is of interest that cocaine 'binging' produces progressive left ventricular dysfunction,¹⁹⁰ lending further support to the reports of α_1 -adrenoceptor modulation of cardiomyocyte hypertrophy, cardiomyocyte necrosis, and progressive ventricular remodeling.¹⁹¹

In the living patient, the diagnosis of coronary vasoconstriction depends on the elucidation of certain clinical variables during the acute episode (i.e., transient ST-segment changes), or by the reproduction of clinical symptoms at a later stage by a controlled provocative clinical trial.^{192,193} Ultimately, the diagnosis in the living patient can be confirmed by visualization of transient constriction during coronary angiography.^{194,195}

For a number of years, Factor and his coworkers had observed focal alterations in coronary artery medial smooth muscle cells studied at autopsy from patients with presumptive coronary spasm, and which has been documented to be a primary cause of transient coronary obstruction in patients with atypical angina pectoris^{196,197} and acute myocardial infarction.¹⁹⁸⁻²⁰⁰ In the postmortem material examined by Factor, the observed smooth muscle changes resembled contraction bands. Accordingly, these workers designed a study to evaluate the frequency of coronary contraction bands in routine autopsy material, and to correlate their presence or absence with any clinical or pathological features that might suggest the development of coronary vasoconstriction.²⁰¹

In this latter study, these workers specifically elected to analyze coronary arteries in an unselected group of patients dying without myocardial infarction and without clinically recognised coronary artery spasm. On completion of this study, these workers found contraction bands in 47 (74.6%) of their 63 cases, concluding that medial smooth muscle

contraction bands were markers of terminal antemortem coronary artery vasoconstriction. More significantly, this study demonstrated the apparent correlation of contraction bands with terminal conditions associated with elevated levels of catecholamines.

While contraction bands have been demonstrated in the media of coronary arteries in association with vasoconstriction as well as in myocardium in association with elevated levels of catecholamines, they have also been described in the myocardium in shock as well as reperfusion injury following myocardial infarction.²⁰² In the myocardium they are also known as zonal lesions and comprise opaque transverse contraction bands within a cardiomyocyte close to an intercalated disc. They are accompanied by shortening of the sarcomere, fragmentation of the Z bands, distortion of the myofilaments, and, under the electron microscope, mitochondrial displacement away from the intercalated disc.

A study of myocardial changes associated with both cardiogenic shock following myocardial infarction and noncardiogenic shock conducted at Harvard nearly forty years ago revealed an interesting pattern of myocyte necrosis differing from that seen in cases of myocardial infarction without shock. This pattern comprised small foci of myocyte necrosis throughout the left and right ventricles varying from a few tenths to 3 mm in diameter. The lesions were often surrounded by vacuolated myocytes and, while neutrophils were uncommon in the lesions, macrophages were present. In addition, some of the most extensive focal necroses occurred in patients with shock in the absence of exogenous catecholamine administration.²⁰³ Both older and more recent studies have shown that shock states with norepinephrine release can produce focal cardiomyocyte necrosis (in addition to left ventricular dysfunction) in the absence of other exogenous factors in a variety of experimental and clinical conditions.²⁰⁴⁻²¹¹

Morphological coincidences between the apoptotic changes observed in the heart and those observed in neural and other tissues

A particularly pronounced morphological change noted in the study was central clearing of the nucleus with peripheral margination and condensation of chromatin along an undulating nuclear membrane. This apoptotic change is of especial interest in the light of a similar disturbance occurring in cortical neurons. In these latter cells, the Nissl substance disappears from the centre of the cell and becomes concentrated at the cellular periphery. While these neuronal changes have been regarded by neuropathological investigators as a nonspecific response to injury, it has nevertheless been observed in many subjects who have general circulatory failure and some degree of hypoxia. Hyperchromaticity of the nuclei of endothelial cells has similarly been described in the same clinical setting.²¹²

Electron microscopic studies of cardiomyocytes have described mitochondrial enlargement and swelling with increase in the intracrystal space in sympathomimetic agent-induced myocardial injury.²¹³ As previously indicated, studies have shown that norepinephrine stimulates hypertrophy of cardiomyocytes thus providing an additional background for the myocardial nuclear changes of hypertrophy seen after head injury with its ensuing catecholamine release.

Further electron microscopic findings that have been observed in cardiomyocytes following sympathomimetic agent-induced myocardial injury are dilatation of the sarcoplasmic reticulum as well as swelling and dilatation of the endoplasmic reticulum.²¹⁴ These electron microscopic findings have also been noted in studies of ischaemic neurons.²¹⁵⁻²²³ In hepatocytes subjected to hypoxia, including that of acute

cyanide poisoning,²²⁴ cytoplasmic microvacuolation has been described similar to that observed in the cardiomyocytes in the study. As seen on electron microscopy, this vacuolation is associated with severe mitochondrial changes and sarcoplasmic dissociation. Similar changes have also been described in the epithelial cells of the thyroid, pituitary, adrenal, and in some parietal cells of the stomach mucosa in subjects exposed to high-altitude hypoxia.²²⁵

The changes observed in the cardiomyocytes in the study are therefore intriguing on several counts: i) it is feasible that the nuclear and cytoplasmic changes in the cardiomyocytes represent, as in cortical neurons and other cells, an episode or element of hypoxia (in the case of cardiomyocytes following on brain injury) or ischaemia following the effect of catecholamines on the myocardium and manifesting as apoptotic cell death; ii) it appears that the nuclei of cells of differing embryological cell lines can and do respond to insults within a very short time of noxious stimuli and, apparently, in a remarkably similar manner. The early onset of nuclear change observed within 20 minutes of injury in the study is analogous to the time span observed in primates in which neuronal alterations were noted within 20 minutes after an episode of controlled hypoxia while astrocytic swelling was noted as early as 10 minutes after death,²²⁶ and; iii) analogous to the changes occurring in neurons following hypoxia in which early neuronal alterations are followed by progressive loss of nerve cells and eventually by a gliomesodermal reaction, so too were the early cardiac histopathological changes followed by a sequential and similar series of reactions.

As indicated, twelve of the subjects in the study were noted to have increased heart weights i.e., cardiac hypertrophy. From a pathological viewpoint this is a potentially very important aspect since ischaemic heart disease, dilated cardiomyopathy or hypertensive left ventricular hypertrophy may in themselves lead to cardiac myocytolysis. This latter aspect is of particular importance in view of the fact that histopathological examination of endomyocardial biopsies or of hearts of patients with dilated cardiomyopathy at autopsy reveals only vague and nonspecific changes and there may be no significant alterations in as many as 25 per cent of the cases.^{227,228} Furthermore, the coronary arteries in patients with dilated cardiomyopathy are most often normal or have minimal to moderate atherosclerosis compatible with the patient's age. However, the hearts in dilated cardiomyopathy patients are always increased in weight, in some cases up to 900 g. while in 30 of the 42 subjects in the study (71.4%) the hearts were of normal weight tending to exclude a dilated cardiomyopathy or hypertensive left ventricular hypertrophy as the cause of the myocytolysis noted.

With regard to the question of ischaemic heart disease being a cause of or contributory factor to the focal myocytolytic changes noted, none of the subjects in the study displayed a significant degree of coronary artery narrowing or atheromatous vaso-occlusive disease. This included case no. 42, that of a 66-year-old black male with a post-injury survival period of five-and-a-half months. This latter finding is in keeping with the generally low incidence of atheromatous coronary artery disease noted in South African black males in comparison with their white counterparts. The coronary arteries had, as indicated, been examined by means of transverse incisions at 3 to 4 mm intervals throughout the major coronary arteries

with individual transverse incisions being then sequentially linked by longitudinal cuts using fine scissors. This latter methodology can, according to most authors, provide adequate data for clinico-pathological correlation.

The significance of the florid myocytolytic lesion and
its evolution and progression to fibrosis-----

Apart from the morphologically distinctive centrifugal nature of the myocytolytic lesion (which manifested as peripherally extending areas of myocytolysis), two further features emerged: i) the progressive nature of the lesion creating a spectrum of increasingly more prominent myocytolysis eventuating and culminating in formation of the so-called 'florid lesion', a term not heretofore used in the literature to describe the fully fledged lesion, and; ii) progression and evolution of this latter lesion to areas of post-myocytolytic stromal collapse fibrosis, more mature fibrosis, well-defined and more discreet areas of fibrosis and, finally, diffuse fibrosis. The failure to clearly differentiate the early developmental stages of focal myocytolysis from the later fully evolved lesion accounts for the discrepancies in the time of onset and evolution of this process noted by different authors as well as a lack of descriptive standardization of this important lesion in the literature.

The findings, therefore, of evolution and progression of the lesions of focal myocytolysis through stromal condensation and collapse eventuating in myocardial fibrosis is of particular significance in the light of studies conducted by Rajs who specifically investigated the relationship between cranio-cerebral injury, subsequent myocardial fibrosis, and

cardiac failure.^{229,230} In these latter studies, all patients had sustained craniocerebral injury, all showed similar histopathological changes in the myocardium to those seen in the present study and all patients surviving beyond 21 days after injury showed evidence of myocardial fibrosis. This included an eight-year-old boy who had survived for seven years after a head injury and in whom death was attributed to acute myocardial insufficiency as a consequence of myocardial fibrosis. The paediatric age group of the patient is of relevance in the light of a report of transient ventricular septal hypertrophy in the first year of life associated with neonatal brain injury,²³¹ as well as of reports of the production of electrocardiographic abnormalities following head injury in children alluded to in chapter six. The report of ventricular septal hypertrophy in the paediatric age group is thus intriguing on two counts: i) the alterations in nuclear morphology found in the present study which, in addition to representing apoptotic cell change are, as previously indicated, a paracrine and autocrine-mediated stress-induced cardiac adaptive and maladaptive response, and; ii) left ventricular hypertrophy has been known to have the potential of developing over the short time span of a few weeks.²³² It is of interest that subarachnoid haemorrhage can, in this context, not only be associated with abnormal myocardial perfusion and reversible left ventricular wall motion abnormalities,²³³⁻²⁴⁷ but that the catecholamine 'storm' characterized by copious release of norepinephrine from cardiac receptor sites during acute cerebral insults is associated with myocardial damage, as reflected in a rise of cardiac enzymes (CPK-MB),²⁴⁸ cardiac troponin I (cTnI) elevation,²⁴⁹⁻²⁵³ left ventricular wall motion abnormalities,²⁵⁴⁻²⁶³ myofibrillar degeneration and contraction band necrosis on light microscopy, and subendocardial injury.²⁶⁴⁻²⁶⁷

As a corollary, and while this is a very different condition, it is of interest that many of the changes observed in the study such as hyperchromaticity of nuclei, alterations in nuclear morphology, prominent interstitial and intracellular oedema, focal myocytolysis and myocardial fibrosis have also been described in muscular dystrophy,^{268,269} with fatal cardiac arrhythmias and sudden death occurring as a result of fibrotic involvement of the Purkinje conducting system.²⁷⁰

The present study thus differs from that of Galiñanes, Smolenski and Hearse previously alluded to in that it raises the issue of long-term impairment of cardiac contractile performance rather than that of the acute impairment occurring in the brain death period immediately preceding cardiac transplantation and which, as shown by these workers, is treatable. Several studies have shown that not only can cardiac myocytolysis in its acute form in humans contribute to or cause death when sufficiently extensive,²⁷¹⁻²⁷⁴ but that brain injury-induced myocardial histopathological change carries the potential of producing myocardial fibrosis, long-term myocardial insufficiency and eventual cardiac failure.²⁷⁵⁻²⁷⁸ Rajs has stated that: 'It is evident that a relationship exists between acute lesions of the myocardium and severe trauma or craniocerebral injury. These lesions may give rise to cardiac hypertrophy and myocardial fibrosis i.e., cardiomyopathy resulting in cardiac insufficiency and death.'

Like the histopathological lesions in the Rajs and other studies,^{279,280} The myocardial lesions in this study appear to reflect different stages in the evolution, progression and development of myocardial lesions following head injury, the latter being the reason for admission to hospital in all subjects in the study. Furthermore, apart from the

two subjects who had only survived for 20 minutes after injury (cases 1 and 2 who demised in the emergency room), all other subjects had been admitted to the neurosurgical ward and had remained there until demise in a comatose state sustaining no further injuries or disease processes other than a terminal bronchopneumonia, a common accompaniment and concomitant of terminal vegetative states.

Conclusion

In view of the statistically significant relationship that was found to exist between craniocerebral injury on the one hand and myocardial replacement fibrosis on the other the latter appearing to have progressively evolved from earlier foci of myocytolysis through post-myocytolytic stromal condensation and stromal collapse fibrosis followed by a more mature stage of scarring it is felt that in those cases with a history of antecedent craniocerebral injury the possibility of long-term myocardial insufficiency and impairment of cardiac contractile function on the basis of myocardial fibrosis should be considered. This latter possibility should be borne in mind both in long-term survivors of head injury and in cardiac transplant recipients who, of necessity, acquire donor hearts from head injured brain dead victims.^{281,282}

It is suggested, therefore, that clinicians treating long-term survivors of head injury, in particular young survivors, be aware of this potential complication and that follow-up, surveillance and monitoring of the clinical cardiovascular status be undertaken routinely and as a standard procedure in these patients. With regard to cardiac transplantation patients in whom, as indicated, most donor hearts have been harvested from head injury victims,²⁸³ the

possible advent of cardiac decompensation on the basis of myocardial fibrosis should be considered, particularly in view of the fact that myocardial scarring and replacement fibrosis has been shown to predominantly affect and involve the left ventricle and its papillary muscles. These areas represent the most actively contractile part of the heart subjected to the greatest volume and hence the correspondingly greatest contractile demands. It is of significance in this regard that a study by Schlesinger and Reiner as far back as 1955 showed focal myocytolysis to be more common in the left ventricle than in the right,²⁸⁴ and with later studies confirming the morphological appearance of the apoptotic lesion.²⁸⁵

Future pharmacological interventions and early institution of appropriate therapeutic modalities may possibly modify beta-adrenergic signal transduction as well as the histopathological lesions of catecholamine-mediated myocardial injury and its progression to myocardial fibrosis enlarging thereby the potential cardiac transplantation donor pool and, more importantly, enhancing patient survival and quality of life.

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UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Division of the Deputy Registrar (Research)

COMMITTEE FOR RESEARCH ON HUMAN SUBJECTS (MEDICAL)

Ref: R14/49 Moar

CLEARANCE CERTIFICATE

PROTOCOL NUMBER M00/07/14

PROJECT

Craniocerebral Injury & Histological Changes
Observed Within The Myocardium

INVESTIGATORS

Dr JJ Moar

DEPARTMENT

Dept of Forensic Medicine, Gauteng Dept of Health

DATE CONSIDERED

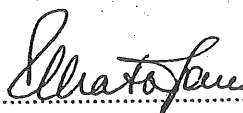
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DECISION OF THE COMMITTEE *

Approved unconditionally

DATE 00/07/31

CHAIRMAN.....



(Professor P E Cleaton-Jones)

* Guidelines for written "informed consent" attached where applicable.

c c Supervisor: Dr E Klug

Dept of Cardiology Department, Johannesburg Hospital

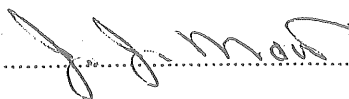
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DECLARATION OF INVESTIGATOR(S)

To be completed in duplicate and ONE COPY returned to the Secretary at Room 10001, 10th Floor, Senate House, University.

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we guarantee to ensure compliance with these conditions. Should any departure to be contemplated from the research procedure as approved I/we undertake to resubmit the protocol to the Committee.

DATE 19/03/01 **SIGNATURE**



PROTOCOL NO.: M 00/07/14

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES